Edge Hill University

The Effects of Gastro-Resistant Sodium Bicarbonate Supplementation on Markers of Acid-Base Balance, Gastrointestinal Symptoms and Exercise Performance

By

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Abbreviations

ηp^2	Partial eta-squared
$\dot{V}O_{2peak}$	Peak oxygen uptake
$[Cl^-]$	Chloride ion concentration
$[H^+]$	Hydrogen ion concentration
$[K^+]$	Potassium ion concentration
$[La^-]$	Lactate ion concentration
$[Na^+]$	Sodium ion concentration
ATP	Adenosine triphosphate
AU	Arbitrary units
CI	Confidence interval
Cl^-	Chloride ion
g	Hedge's g
GI	Gastrointestinal
GI	Gastrointestinal
H^+	Hydrogen ion
HCO_3^-	Bicarbonate ion
HCO_3^-	Bicarbonate ion
K^+	Potassium ion
La^-	Lactate ion
Na^+	Sodium ion
$NaHCO_3$	Sodium bicarbonate
pH	Potential hydrogen
ROF	Rating of perceived fatigue
RPE	Rating of perceived exertion

RPE-L	Localised rating of perceived exertion
SpO_2	Oxygen saturation
TT	Time trial
W	Watts

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Abstract

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Abstract

Sodium bicarbonate ($NaHCO_3$) is an extracellular buffering agent and nutritional ergogenic aid that can improve high-intensity exercise performance. Based upon prior research, $NaHCO_3$ supplementation can improve markers of exercise metabolism, recovery and performance due to its ability to augment extracellular buffering capacity and delay exercise-induced fatigue. Nevertheless, gastrointestinal (GI) side-effects are widely reported following $NaHCO_3$ ingestion, which might deter athletes from using $NaHCO_3$ or confound the benefits of supplementation. When combined with gastric acid, $NaHCO_3$ produces excess carbon dioxide that can lead to various GI symptoms, including stomach cramps, bowel urgency and diarrhoea. Bypassing the stomach presents a novel strategy to prevent GI symptoms, which may be achieved using gastro-resistant capsules. Gastro-resistant $NaHCO_3$ could therefore offer a convenient and practical strategy to reduce GI symptoms associated with $NaHCO_3$ supplementation, whilst improving exercise performance. Nevertheless, the efficacy of enteric-coated $NaHCO_3$ supplementation has yet to be investigated. The subsequent investigations were conducted to further explore the effect of gastro-resistant $NaHCO_3$ supplementation on GI symptoms, acid-base responses and high-intensity exercise performance in recreationally trained and trained males. Given that delayed-release capsules are suggested to bypass the stomach and reduce GI symptoms caused by acidic-sensitive compounds (such as $NaHCO_3$), this was investigated in Study 1. Compared with the solution, delayed-release $NaHCO_3$ reduced the prevalence and severity of GI symptoms and slowed metabolic alkalosis, although the overall acid-base responses (e.g. peak blood [HCO_3^-] and pH) were similar. Nevertheless, it was unclear whether these affects were related to encapsulation or the gastro-resistant properties of the capsule. Based on these findings, it could also be suggested that enteric-coated $NaHCO_3$, which are more gastro-resistant than delayed-release capsules, could further reduce GI symptoms. Study 2 therefore investigated the effects of different gastro-resistant capsules

(i.e. delayed-release and enteric-coated) compared with a standard capsule (i.e. gelatine) on GI symptoms and acid-base responses. Gastro-resistant capsules mitigated GI symptoms compared with the gelatine capsule, suggesting that bypassing the stomach is an efficacious strategy to reduce GI symptoms following $NaHCO_3$ ingestion. Whilst enteric-coated $NaHCO_3$ resulted in the fewest (and less severe) GI symptoms, acid-base responses were blunted with this ingestion form. Study 3 therefore investigated whether enteric-coated $NaHCO_3$ could still improve high-intensity exercise performance. Enteric-coated $NaHCO_3$ improved exercise performance during a 4 km cycling time trial in trained males, although the magnitude of effect was lower than with gelatine capsules. Collectively, these studies demonstrate that bypassing the stomach is an efficacious strategy to reduce GI symptoms with $NaHCO_3$ supplementation, which can be achieved using gastro-resistant capsules. gastro-resistant $NaHCO_3$ can therefore be used to mitigate GI symptoms, although these findings also suggest they should not replace standard ingestion forms in those who can tolerate them. Based on the available evidence, practical recommendations are provided for athletes which will develop as further research is conducted in this area. With this knowledge, further explorations into different gastro-resistant ingestion forms can be made, which in turn, can refine this novel and practical ingestion strategy.

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Chapter 1: General Introduction

1.1 Introduction to research area

Human exercise performance that relies upon anaerobic metabolism has limited capacity (MacLaren, 1989; Baker et al. 2010). Despite the conscious will of the athlete to drive mechanical work rate, performance inevitably falls and eventually ceases (MacLaren, 1989). This concept of fatigue has intrigued scientists for decades (Mosso, 1915, Di Giulio, Daniele and Tipton, 2006) and attempts to underpin the mechanisms of exercise-induced fatigue and delay its deleterious effects on exercise performance has been the focus of considerable research. To date, there is currently substantial evidence (Maughan et al. 2018) that both exercise training and nutritional intervention can delay exercise-induced fatigue.

Intense exercise provides a significant challenge to maintain physiological homeostasis in humans. Exercise demanding high rates of adenosine triphosphate (ATP) relies upon anaerobic glycolysis, although its capacity is limited, at least in part, due to the accumulation of hydrogen ions. While considerable debate remains as to the mechanisms of fatigue during exercise, increases in hydrogen ion concentration ($[H^+]$) are regarded as contributory. Exogenous buffering systems have evolved in humans to remove excessive metabolites and thus maintain $[H^+]$ within extremely narrow limits. In the absence of this mechanism, deviations to homeostasis would lead to catastrophic failure of the entire organism. In the context of sport, metabolic acidosis is considered at least partly attributable to impaired skeletal muscle contraction and reductions in exercise performance.

Multiple attempts to delay the deleterious effects of fatigue on performance have been made, both in relation to exercise training and nutritional strategies. Research studies indicate that exercise training can induce significant improvements in exogenous buffering capacity, that can translate to improved exercise performance (Laursen and Jenkins, 2002). Of the nutritional strategies employed, acute sodium bicarbonate ($NaHCO_3$) loading has been found

to have a profound effect on exercise performance. In a meta-analysis, $NaHCO_3$ supplementation was associated with moderate (1.7 ± 2.0%) improvements during a single 1-minute sprint, increasing by ~ 0.5 ± 0.5% for a larger dose or five additional sprint bouts.

While the ergogenic efficacy of $NaHCO_3$ ingestion is well established during intense (anaerobic) exercise, oral administration is associated with gastrointestinal (GI) side-effects (Cameron et al. 2010, Kahle et al. 2013, Saunders et al. 2014a), including stomach bloating and diarrhoea. Gastro-resistant compounds can reduce GI side-effects associated with the oral administration of acid-sensitive compounds, although this has yet to be applied in the context of sport.

1.2 Structure of thesis

The overall purpose of this thesis is to explore the role of gastro-resistant $NaHCO_3$ on acid-base balance, GI symptoms and exercise performance. Firstly, a review of the current literature will examine the factors modulating the effect of $NaHCO_3$ supplementation on exercise performance and the potential application of gastro-resistant ingestion forms (Chapter 2). The subsequent chapter will detail the general methods that will be employed to answer the respective research questions, including the procedures that will remain consistent across all studies that compose this thesis (Chapter 3). Next, a series of experimental studies will be conducted that will address the research aims (Chapters 4, 5 and 6). Finally, the overarching conclusions will be discussed in relation to existing bodies of work and practical recommendations will be provided for athletes and coaches (Chapter 7).

1.3 Aims

Research has been presented which indicates that acute $NaHCO_3$ loading can improve exercise performance, although this can also result in GI side-effects. The neutralisation of gastric acid

is shown to increase carbon dioxide production, which in turn, can result in GI symptoms including stomach bloating, vomiting and diarrhoea. Consequently, this can reduce the performance-enhancing effects of $NaHCO_3$ supplementation and in some cases, may even be ergolytic. Gastro-resistant $NaHCO_3$ may therefore present a novel strategy to mitigate GI symptoms whilst improving exercise performance. However, there is no empirical evidence to suggest that ergogenic doses of gastro-resistant $NaHCO_3$ can reduce GI symptoms and subsequently improve exercise performance (~ 300 $mg \cdot kg^{-1}$ body mass). This thesis will therefore address the following research aims.

1. Determine the prevalence and severity of GI symptoms with $NaHCO_3$ supplementation (addressed in Studies 1, 2 and 3)
2. Explore the role of gastro-resistant capsules on GI symptoms and acid-base responses (addressed in Studies 1 and 2)
3. Investigate the efficacy of gastro-resistant $NaHCO_3$ to improve high-intensity exercise performance (addressed in Study 3)

Chapter 2: Literature Review

2.1 Determinants of exercise fatigue and performance

Human exercise performance is determined by numerous physiological, psychological and biomechanical factors (Joyner and Coyle, 2008) that are relative to the specific demands of the task. Although physiological exercise capacity does not determine exercise performance per se, its superiority is considered to enhance the potential for better exercise performance. For almost a century, Exercise Physiologists have studied the physiological determinants of human performance, particularly in relation to fatigue and its origins. In a series of studies conducted by Hill and colleagues in the early 1920's (Hill and Lupton, 1923, Hill, Long and Lupton, 1924a, 1924b, 1924c), the concept of maximal oxygen consumption ($\dot{V}O_{2max}$) was introduced, proposing that there was an inter-individual upper limit to oxygen uptake which was limited by the cardiorespiratory systems ability to transport oxygen (O_2) to skeletal muscle. The authors suggested that a high $\dot{V}O_{2max}$ was a significant determinant of endurance performance and was regulated by cardiac output and an elevated arteriovenous O_2 difference. Elite endurance athletes have indeed reported superior $\dot{V}O_{2max}$ values due to substantial adaptations to aerobic exercise training (Costill et al. 1976). Later research (Bassett and Howley, 2000) suggested that the ability to sustain higher work rates for longer durations is associated with superior O_2 efficiency and exercise performance. Indeed, maintaining aerobic metabolism as the predominant energy supplier was found to be associated with a delay in fatigue, and performance could be sustained for prolonged periods. When work rate exceeded that which could be supplied by aerobic metabolism, anaerobic pathways became predominant and the individuals were deemed to have reached their anaerobic threshold (Joyner and Coyle, 2008). Anaerobic threshold is suggested to represent the work rate at which a dynamic equilibrium is reached between metabolite production and removal (Billat et al. 2003), beyond which substantial deviations to bodily homeostasis occur, and thus exercise performance falls

dramatically. Enhanced removal of these metabolites or a greater tolerance to their accumulation is as suggested, essential to delay the effects on performance.

The origins and purpose of fatigue are controversial and intensely debated topics, of which a consensus has yet to be reached. Scientists have proposed both peripheral and central origins (Gandevia, 2001, St Clair Gibson and Noakes, 2004) of fatigue. Peripheral origins of fatigue suggest that fatigue occurs within, or distal to the neuromuscular junction, thereby limiting external work that can be mechanically performed. Central mechanisms of fatigue propose that work rate and fatigue are regulated by the brain through central motor output. Sensory information obtained through feedforward mechanisms is used to adjust central motor output to avoid catastrophic deviations to homeostasis that would otherwise be induced by exercise (St Clair Gibson and Noakes, 2004). Two central mechanisms have been proposed, including the central governor model, a largely subconscious phenomena, and the psychobiological model, proposing that conscious perception of effort adjusts central motor output (St Clair Gibson and Noakes, 2004, Marcora and Staiano, 2010). While evidence exists to support these models of fatigue, it is currently accepted that both central and peripheral factors contribute to the onset of fatigue (Thomas et al. 2014).

2.2 Origins of fatigue

Exercise-induced fatigue is commonly defined as the impaired ability to produce muscular force during exercise, often accompanied with an increased perception of effort (Gandevia, 2001, Enoka and Stuart, 1985). Fatigue can be attributed to various processes along the motor pathway that are broadly split into central and peripheral origins (Amann, 2011). Central fatigue suggests that declines in muscle force reside within the central nervous system (i.e. brain and spinal cord), including changes in neurotransmitter activity, motor cortical output and muscle efferent and afferent feedback (Gandevia, 2001). Furthermore, the central nervous

system preserves the development of peripheral fatigue, primarily through increases in ventilation, cardiac output and muscle blood flow during exercise (Amann and Calbet, 2008). In contrast, peripheral fatigue attributes the declines in muscle force to processes across the neuromuscular junction and sarcolemma (Gandevia, 2001). This includes a lack of excitation-contraction coupling and metabolite accumulation (e.g. H^+) which perturb acid-base balance (Allen et al., 2008). Fatigue is therefore the result of contributions from both central and peripheral processes, which are largely determined by the exercise task (e.g. intensity). Fatigue that is induced during high-intensity, short-duration exercise typically has a substantial peripheral component, whereby the contributions from the central nervous system are relatively low (Schillings et al. 2003). Nevertheless, central fatigue becomes predominant as the exercise duration increases or during submaximal but prolonged exercise intensities (Smith et al. 2007, Sogaard et al. 2006). The mechanisms to explain how this occurs, however, are complex, controversial and currently widely debated.

2.2.1 Metabolic acidosis and fatigue

The fuels used in anaerobic exercise (e.g. 100 m sprint) differs from those used in aerobic exercise (e.g. half marathon) and is largely determined by the intensity and duration of exercise (Baker et al. 2010). During high-intensity exercise, the demand for ATP is high, and therefore requires a high rate of ATP production from fuels other than stored ATP and phosphocreatine (Gastin, 2001).

Through the process of glycolysis, one glucose molecule is broken down to form two pyruvate molecules and H^+ (MacLaren and Morton, 2012). Given the high oxygen and energy (i.e. ATP) demand during high-intensity exercise, pyruvate remains within the cytoplasm and converts to lactate – a process termed anaerobic glycolysis. This series of ten enzyme-catalysed reactions is shown in Figure 2.1.

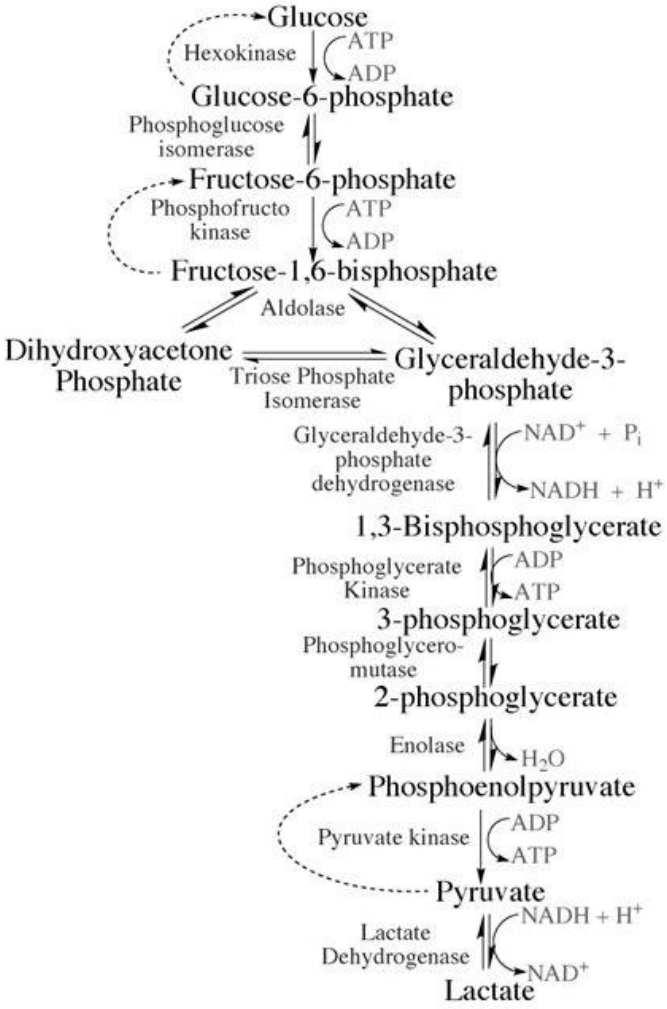

Figure 2.1. Breakdown of glucose to form pyruvate, lactate and H^+ during anaerobic glycolysis (adapted from MacLaren and Morton, 2012).

Muscle fatigue is thought to be at least partly the result of a decrease in muscle pH due to the accumulation of H^+ during anaerobic glycolysis. Whilst the mechanisms responsible for the onset of muscle fatigue across are equivocal (Fitts, 2016; Westerblad, 2016), reductions in muscle pH are associated with simultaneous declines in muscle excitability (Cairns & Lindinger, 2008), contractility (Spriet et al. 1985) and exercise performance (Raymer et al. 2004). Nevertheless, these causes of muscular fatigue are currently contested, with research suggesting a negligible role of pH on force generation and recovery (Westerblad, 2016). It is

well established enzymes have an optimal pH and that acidosis can result in reduced glycolytic enzyme activity (MacLaren et al. 1989), suggesting at least some role of acidosis on muscle fatigue. This is further supported by the observation that inducing acidosis decreases high-intensity exercise performance (Correia-Oliveira et al. 2017), whereas alkalosis can improve it (Carr et al. 2011c).

2.3 Acid-base balance

Acid-base balance governs the acidity or alkalinity of a solution, which is largely determined by $[H^+]$ and is more often expressed as the logarithm termed potential hydrogen (pH) in Equation 1.

$$(1)\ pH = -\log_{10}[H^+].$$

Given that pH is the negative logarithm of $[H^+]$, there is inverse relationship between blood $[H^+]$ and pH. Elevations in $[H^+]$ thereby result in a lower pH, a state commonly termed acidosis. Given that small changes in $[H^+]$ disturb protein and membrane function, bodily pH is regulated within very narrow limits. Despite disagreement as to normal pH reference values, blood pH is considered to fluctuate between 7.35 and 7.45 AU to facilitate metabolic and physiological processes (Berend, 2013). Biochemical sources of H^+ include intermediary and end-products of metabolism such as glycolysis, specially under anaerobic conditions (i.e. anaerobic glycolysis). Exercise therefore provides a substantial endogenous source of H^+ that is relative to the energetic demands of the activity. Consequently, humans have developed intricate mechanisms to maintain pH within the various bodily compartments, including that of the chemical buffer, respiratory and renal systems (Poupin et al. 2012).

2.4 Body buffer systems

Buffer systems primarily exist to regulate acid-base balance, and work to maintain dynamic equilibrium between an un-dissociated weak acid (HA) and its conjugate base (A^-), as depicted in Equation 2.

$$(2)\ HA = [H^+] + A^-$$

Should $[H^+]$ rise, they are combined with the weak base to form additional weak acid therefore lowering $[H^+]$. Conversely, should there be a fall in $[H^+]$, more may be liberated from the weak acid. Both reactions operate to return $[H^+]$ and therefore pH, to within normal limits. Metabolic acidosis is the result of substantial elevations in $[H^+]$ and occurs when blood pH falls below ~7.35 AU, whereas metabolic alkalosis occurs when $[H^+]$ decreases and blood pH exceeds ~7.45 AU. In both cases, an effective system is required to mediate acid-base balance and avoid deleterious effects on the human body. Three basic mechanisms exist to maintain pH homeostasis in man, including physicochemical buffering and respiratory and renal compensation.

Extracellular buffering occurs through transporting protons (e.g. H^+) out of the muscle and into the blood. Across the muscle membrane, monocarboxylate transporters 1- and 4 are responsible for maintaining muscle pH (Bishop et al. 2006) through the translocation of lactate and H^+ by the lactate/H^+ transporter. Given that monocarboxylate transporter 4 is expressed in high quantities in glycolytic type IIb muscle fibres and those with low mitochondrial density (Bishop et al. 2006), the bicarbonate buffer system plays a significant role in extracellular buffering (Poupin et al., 2012).

2.4.1 Bicarbonate buffer system

Physicochemical buffering works instantaneously to attenuate fluctuations in pH. Elevations in pH stimulates proteins, haemoglobin, phosphates or bicarbonate to undergo chemical reactions that prompt their disposal (Atherton, 2009, Poupin et al. 2012). Of the physicochemical mechanisms, the bicarbonate buffer system is fundamental to maintaining acid-base balance, accounting for 86% of total buffering capacity in extracellular space, with protein and haemoglobin responsible for the remaining 14% (Poupin et al. 2012). Within intracellular space, bicarbonate is accountable for 34% of overall buffer capacity, whereas proteins and phosphates have a superior role and are responsible for a combined 66% of total buffer capacity (Atherton, 2009, Poupin et al. 2012).

Bicarbonate buffering regulates blood pH by combining H^+ with HCO_3^- to form carbonic acid (H_2CO_3), which in turn, dissociates to CO_2 and water H_2O. This reversible process is catalysed by the enzyme *carbonic anhydrase* and is expressed in Equation 3.

Carbonic anhydrase

(3) $CO_2 + H_2O \leftrightarrow H_2CO_3 \leftrightarrow H^+ + HCO_3^-$

The abovementioned reaction occurs until a state of dynamic equilibrium is reached, in that elevations in either H^+ or CO_2 drives the reaction in the opposing direction. In this way, rises in CO_2 partial pressure stimulates H_2CO_3 formation which then dissociates to H^+ and HCO_3^-, thus increasing acidity within the solution. Similarly, elevations in $[H^+]$ stimulates H_2CO_3 formation, which dissociates to CO_2 and H_2O thus inducing an alkalising effect. Multidirectional movement of this reaction however is limited, due to the finite availability of $[HCO_3^-]$. Depleting HCO_3^- induces substantial elevations in $[H^+]$ which reflects the upper limit of the bicarbonate buffer system. Bicarbonate buffering can therefore only reduce the

magnitude of pH changes alone. Consequently, the respiratory and renal systems compensate by either removing excess CO_2 or releasing additional HCO_3^- to preserve pH homeostasis, respectively.

2.4.2 Respiratory and renal compensation

Similar to bicarbonate buffering, respiratory compensation is readily available and acts to reduce arterial CO_2 concentrations. Respiratory compensation therefore lessens dependence on bicarbonate buffering to maintain blood pH. This is achieved through a combination of increasing expired CO_2 and a rise in pulmonary ventilation (Atherton, 2009). Given that deoxygenated haemoglobin (venous blood) has greater CO_2 buffering capacity than oxygenated haemoglobin (arterial blood), this promotes venous CO_2 uptake and increases VCO_2 (Rubana and Aulik, 1989). Furthermore, increased $PaCO_2$ detected by peripheral and central chemoreceptors stimulates the vasomotor centre of the medulla to increase respiratory rate and tidal volume (Mitchell et al. 1963). These processes are fundamental during all forms of exercise, when there are substantial elevations in $[H^+]$ and CO_2 (Guyton, 1976). Renal compensation can provide a significant contribution to pH regulation through replenishing bicarbonate stores. Exercise-induced disturbances to acid-base balance are not directly regulated by the kidneys as this system typically functions over several days (Atherton, 2009).

2.5 Sodium bicarbonate

Investigations into skeletal muscle fatigue and exercise performance spans almost a century. Early work (Dennig et al. 1931, Dill, Edwards and Talbot, 1932) speculated that inducing a state of alkalosis could enhance exercise performance, although research on the ergogenic potential of buffering agents was not prominent until the mid-seventies (Jones et al. 1977,

Sutton, Jones and Toews, 1981, McCartney, Heigenhauser and Jones, 1983, Kowalchuk, Heigenhauser and Jones, 1984).

The ingestion of $NaHCO_3$ is now a well-established nutritional strategy that can enhance exercise performance that relies heavily on anaerobic glycolysis (McNaughton et al. 2016). This is currently regarded as high-intensity exercise bouts sustained for 1-10 min (Matson and Tran, 1993), and sports involving repeated sprint bouts over longer durations (30-60 minutes). As an extracellular buffering agent, $NaHCO_3$ helps to maintain pH homeostasis by reinforcing endogenous physicochemical buffering, namely that of the bicarbonate buffer system (Atherton, 2009). Ingestion of $NaHCO_3$ causes a transient increase in circulating HCO_3^- and results in a corresponding increase in blood pH (Costill et al. 1983). By inducing this state of metabolic alkalosis, $NaHCO_3$ helps to maintain intramuscular pH during strenuous exercise, where there is a significant increase in the production of H^+. Given that H^+ can impair skeletal muscle contraction (Fabiato and Fabiato, 1978) and reduce energy turnover through glycolytic pathways (Sahlin et al. 1975), accumulation of H^+ is associated with performance decrements (Fitts, 2016). Generally, it is accepted that elevated levels of circulating [HCO_3^-] enhance efflux of H^+ from the active muscle (Ren et al. 1988; Bangsbo et al. 1995) by the lactate-proton transporters, monocarboxylate transporters 1 and 4 (Mainwood and Worsley-Brown, 1975). However, alternative mechanisms have been proposed including enhanced excitation-contraction coupling and strong ion regulation (Siegler et al. 2016). Fundamentally, there is a delay in the onset of fatigue, which is associated with performance improvements.

2.5.1 Mechanism of action

The psycho-physical basis of fatigue is controversial at least, and the direct mechanisms in which $NaHCO_3$ might evoke an ergogenic effect, are therefore unclear. In addition, empirical research has focused on the impact of $NaHCO_3$ on performance outcomes, with less aiming to

elucidate its potential mechanism. While multiple mechanisms have been postulated, $NaHCO_3$ is repeatedly cited to attenuate intramuscular acidity which contributes to skeletal muscle fatigue (Sahlin, 1992, Fitts, 1994, Knuth et al. 2006, Messonnier et al. 2007, Kent-Braun, Fitts and Christie, 2012). Anaerobic glycolysis yields H^+ as a metabolite, which accumulates in skeletal muscle if production exceeds removal, and reduces intramuscular pH. Muscle biopsies taken after high-intensity exercise to exhaustion reveal intramuscular pH falls to ~6.5 AU from ~7.1 AU at rest (Ahlborg et al. 1972, Costill et al. 1983, 1984), indicating substantial changes to acid-base balance. One mechanism to explain how intramuscular acidosis contributes to muscular fatigue suggests that competition between H^+ and calcium ions (Ca^{2+}) for troponin binding sites impairs skeletal muscle contraction (Donaldson, Hermansen and Bolles, 1978, Fabiato and Fabiato, 1978). Other mechanisms include the inhibition of both phosphocreatine resynthesis (Sahlin, Harris and Hultman, 1975), and glycolytic enzymes such as glycogen phosphorylase and phosphofructokinase (Sutton, Jones and Toews, 1981). Ingestion of $NaHCO_3$ causes a transient but significant rise in blood [HCO_3^-], which is accompanied by an increase of [HCO_3^-] in the extracellular environment. In turn, this steepens the intracellular/extracellular [H^+] gradient and stimulates the lactate/H^+ co-transporters (Hermansen and Osnes, 1972, Mainwood and Worsley-Brown, 1975, Mainwood and Cechetto, 1980, Roth, 1991). Enhanced bicarbonate buffering and efflux of H^+ towards extracellular compartments reduces intramuscular pH, thus attenuating impairments in skeletal muscle contraction and associated exercise performance.

2.5.2 Dose

In order to attain sufficient increases in blood [HCO_3^-], large boluses of $NaHCO_3$ must be orally ingested and are typically prescribed relative to an individual's body mass. While 200 to 300 $mg \cdot kg^{-1}$ body mass are the most commonly ingested doses of $NaHCO_3$, the efficacy of

doses ranging from 100 to 500 mg·kg^{-1} body mass has been examined across the literature (McNaughton 1992a, Stannard et al. 2016). Since the early work of McNaughton (1992), a dose-response relationship is considered to exist between $NaHCO_3$ supplementation and performance, with no further improvements occurring beyond 300 mg·kg^{-1} body mass. Elevations in blood [HCO_3^-] therefore increase relative to the ingested dose, and absolute increases exceeding 6 mmol·L^{-1} are frequently observed for doses of 300 mg·kg^{-1} body mass (Heibel et al. 2018). Increases of this magnitude (i.e. ≥ 6 mmol·L^{-1}) have been suggested to represent a threshold whereby an ergogenic effect is deemed 'almost certain' (Matson and Tran, 1993, Carr et al. 2011b). In some cases, a dose of both 200 and 300 mg·kg^{-1} body mass has demonstrated a comparable performance enhancing effect (Heibel et al. 2016; Stannard et al. 2016).

2.5.3 Ingestion timing

Recently, the timing of ingestion has been recognised to impact the likelihood of observing an ergogenic effect (Heibel et al. 2016; Miller et al. 2016). Typically, $NaHCO_3$ is administered 60 to 90 min prior to exercise (Higgins et al. 2013; Christensen et al. 2014), although recent investigations have further questioned this ingestion strategy. Indeed, current findings propose that when exercise coincides with peak alkalosis, a highly reproducible ergogenic response is observed (Gough et al. 2018). Contrary to prior research (McKenzie et al. 1986), lower doses (200 mg·kg^{-1} body mass) have produced equivalent performance improvements to higher doses (300 mg·kg^{-1} body mass), although this is variable between indidivuals (Gough et al. 2018). However, numerous studies (Jones et al. 2006; Gough at el., 2017b; Sparks et al. 2017) have observed a large degree of inter-individual variability in the time to reach peak alkalosis when examined up to 3 h post-ingestion. In relation to [HCO_3^-] availability, both the time to reach peak [HCO_3^-] and absolute change demonstrate extreme variability between individuals,

although both appear to increase with higher doses (Jones et al. 2016). Across the literature, studies have reported that time-to-peak bicarbonate can occur between 10 to 180 min post-ingestion (Miller et al. 2016; Jones et al. 2016) highlighting the magnitude of the variability. Similarly, absolute change in bicarbonate varies between individuals and may account for equivocal findings regarding ergogenic effects. While significant variability exists between individuals, both time-to-peak bicarbonate and pH demonstrate a high degree of reliability (Gough et al. 2017). Given that the bicarbonate response is deemed more reproducible than pH, current research suggests that ingestion timing should be based on the timing of peak blood [HCO_3^-]. Whilst further research is required to conclude whether exercise performed at peak [HCO_3^-] may indeed optimise $NaHCO_3$ supplementation, there is some evidence to support this ingestion strategy.

2.5.4 Ingestion form

Two different $NaHCO_3$ dosage forms have been used across the literature, including solutions and gelatine capsules, although only one study has made a direct comparison of their effect on acid-base balance and GI-related side-effects (Carr et al. 2011c). Solutions are the most common way of administering $NaHCO_3$, whereby the white crystalline powder is dissolved in water. In some instances, flavoured cordial is added to the solution to enhance palatability and provide a taste-matched placebo. Whilst little attention has been given to how ingestion form may alter the ergogenic response to $NaHCO_3$ supplementation, both ingestion forms (solution and gelatine capsules) have demonstrated performance-enhancing effects. In studies by Bellinger (Bellinger et al. 2012) and Driller (Driller et al. 2012), mean power output improved by a similar degree (~3.1%) when $NaHCO_3$ was ingested with 10 $ml \cdot kg^{-1}$ body mass water 90 minutes prior to 4-minute maximal cycling. Similarly, when the equivalent dose was ingested in gelatine capsules with comparable quantities of water, TTE improved by 2.7% in a study by

Van Mondtfoort (Van Montfoort et al. 2004), demonstrating that both forms of administration can enhance performance. Interestingly, Driller (Driller et al. 2012) reported mild bloating and nausea in 38% participants, whereas Van Mondtfoort (Van Montfoort et al. 2004) reported no such side-effects, although ingestion with gelatine capsules is still associated with GI distress (Sale et al. 2011, Saunders et al. 2014a).

2.5.5 Effects on high-intensity exercise performance

Of the extracellular buffering agents available, $NaHCO_3$ is currently regarded as the most effective, with various narrative reviews (Heibel et al. 2018; McNaughton et al. 2016) and meta-analyses (Carr et al. 2011c) to support this claim. While a significant body of research suggests that $NaHCO_3$ enhances exercise performance, not all studies have reported an ergogenic effect following supplementation (Saunders et al. 2014a; Webster et al. 1993; Kupcis et al. 2012). Equivocal findings may be attributable, at least in part, to the exercise protocol employed in the respective study. As an extracellular buffering agent, the efficacy of $NaHCO_3$ is observed during exercise that induces a performance-limiting degree of metabolic acidosis. Hence, an ergogenic effect should only be observed during exercise that liberates energy (i.e. ATP) under anaerobic conditions. Given that high-intensity, short-duration exercise up to ~ 30 s is not primarily limited by reductions in muscular pH, $NaHCO_3$ has not been found to be beneficial during single exercise bouts of this duration (McNaughton, 1992a). Similarly, endurance-based and steady-state exercise does not induce substantial perturbations to acid-base homeostasis. While some studies suggest that $NaHCO_3$ may improve endurance-based performances (McNaughton, Dalton and Palmer, 1999), most have reported no benefit (Northgraves et al. 2014; Stephens et al. 2002; Freis et al. 2017). The efficacy of $NaHCO_3$ is most notable during single-bouts of high-intensity exercise lasting ~ 1-10 min (Deb et al. 2017, 2018; McNaughton et al. 2016), or during repeated bouts of short-duration (4-90 s) exercise

(Price, Moss and Rance, 2003; Bishop et al. 2004; Bishop and Claudius, 2005; Krustrup et al. 2015; Miller et al. 2016), where there is a significant degree of muscular acidosis (Hermansen and Osnes, 1972; Belfry et al. 2012). Indeed, consistent improvements in performance have been noted during repeated bouts of high-intensity exercise, while intermittent high-intensity sports have also reported similar ergogenic effects (Artioli et al. 2007; Lindh et al. 2008; Siegler and Hirscher, 2010; Tan et al. 2010). In a comprehensive meta-analysis, a moderate (~ 1.7%) performance-enhancing effect was observed for a single 1-min bout of high-intensity exercise, which increased by 0.5% for five additional bouts (Carr, Hopkins and Gore, 2011). However, mean improvements reported with $NaHCO_3$ are highly variable between studies, and multiple factors have been suggested to modulate the individual response (Heibel et al. 2018). Factors such as dose, ingestion timing and training status may all influence individual responses to $NaHCO_3$ supplementation, and increase the variability observed between subjects. Furthermore, there is evidence to suggest that there is a large degree of within-subject variability in the ergogenic response with $NaHCO_3$, which may be affected by adverse side-effects and blood responses (Saunders et al. 2014b). Prior meta-analyses may have therefore underestimated the effect size of $NaHCO_3$, and caution should be taken when interpreting pooled data to assess the efficacy of this supplement.

2.6 Gastrointestinal side-effects

Acute GI distress is a frequently reported side-effect following the oral ingestion of $NaHCO_3$ (Cameron et al. 2010, Kahle et al. 2013, Saunders et al. 2014a) and may arguably be a major determinant of its use among athletes. While various aetiologies have been proposed, the neutralisation of stomach acid is considered the main contributing factor. Indeed, when $NaHCO_3$ enters the stomach, the [HCO_3^-] dissociates and neutralises the highly acidic stomach acid. Given that stomach acid contains hydrochloric acid, the effervescent reaction between

HCO_3^- and hydrochloric acid produces substantial volumes of CO_2 in the stomach. Figure 2.2 illustrates the reaction between HCO_3^- and hydrochloric acid present in the stomach which produces CO_2. Consequently, this can result in abdominal pain, nausea and diarrhoea (Breitkreutz et al. 2007), although symptoms can vary between individuals.

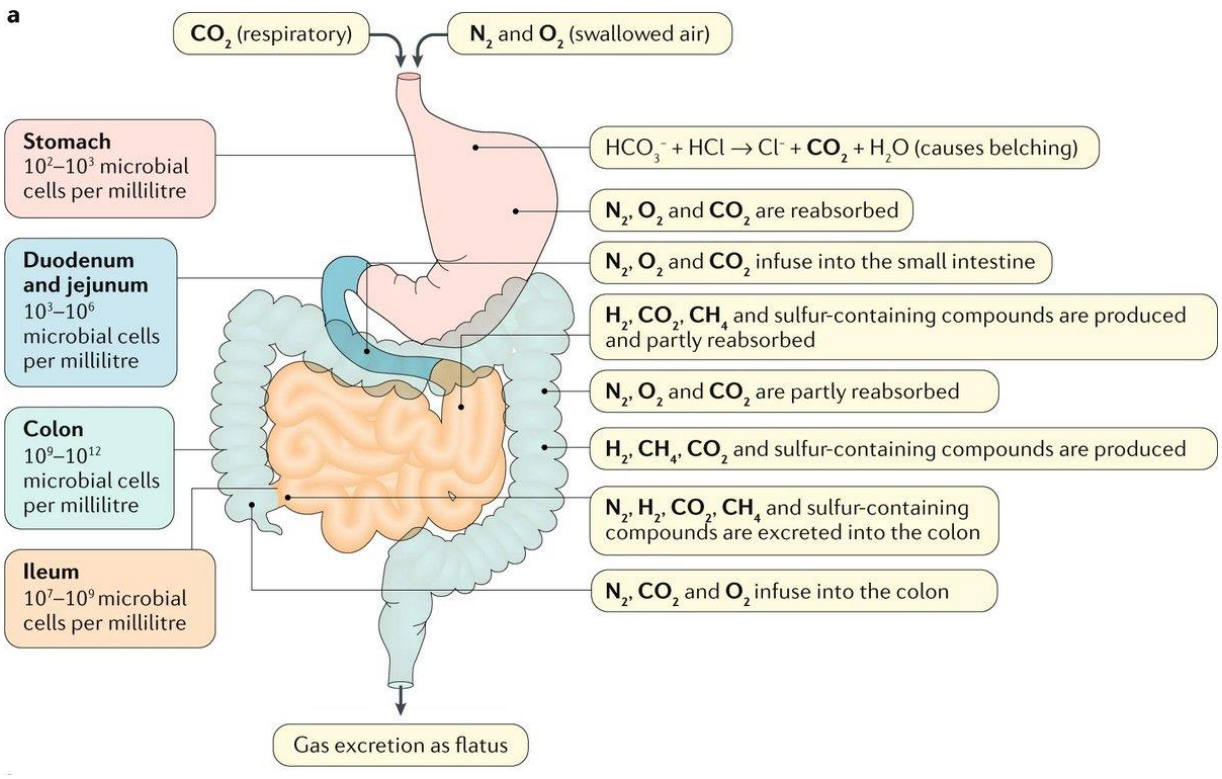

Figure 2.2. Reaction between bicarbonate (HCO_3^-) and hydrochloric acid (HCl) producing CO_2. Adapted from Kalantar-Zadah et al. (2019).

Ergogenic doses (300 mg·kg^{-1} body mass) of $NaHCO_3$ typically results in sodium intakes that exceed dietary reference values (> 2300 mg·day^{-1}). In turn, this can create osmotic fluctuations in the intestinal lumen leading to GI symptoms including diarrhoea (Gisolfi, 1990). As such, GI symptoms may be at least partly due to the high dose of sodium ingested. Nevertheless, the neutralisation of gastric acid is considered the main contributing factor, given that GI side-

effects are far more prevalent and severe following $NaHCO_3$ ingestion compared with sodium chloride (Gough et al. 2018).

2.6.1 Implications of gastrointestinal symptoms

Given that side-effects vary between individuals, this may account for the large intra-individual differences observed in performance. In a study by Saunders (Saunders et al. 2014a), an ergogenic effect was only detected when subjects who experienced GI distress, including moderate to severe stomach cramps and diarrhoea, were removed from statistical analyses. The remaining subjects reported a 4.7% improvement in total work done during a supramaximal (110% peak power) cycle bout to exhaustion. Another explanation for large intra-individual differences in performance may be related to symptom tolerance. Research trials have reported that some individuals can undergo performance enhancing effects of acute $NaHCO_3$ loading despite symptoms of GI distress (McNaughton, 1992, Miller et al. 2016), whereas others cannot (Cameron et al. 2010, Saunders et al. 2014a). Discrepancies between studies may relate to symptom severity, however some studies did not provide sufficient detail relating to side-effects and direct comparisons cannot be made. In practice however, GI distress still presents an issue for athletes, and multiple strategies to reduce its negative implications have been researched (McNaughton and Thompson, 2001; Mueller et al. 2013; Sale et al. 2011, Saunders et al. 2014a, Saunders et al. 2014b).

2.6.2 Strategies to reduce gastrointestinal symptoms

Multiple dosing strategies have been investigated to minimise the incidence and severity of GI symptoms, including chronic (McNaughton and Thompson, 2001), multi-day (Mueller et al. 2013) and split-dose (Sale et al. 2011, Saunders et al. 2014a, Saunders et al. 2014b) administration. Chronic and multi-day administration provides excessive quantities of sodium

which present other side-effects, whereas split dosing protocols may still induce GI distress (Sale et al. 2011, Saunders et al. 2014a, Saunders et al. 2014b). Given that larger doses appear to increase the incidence or worsen the severity of GI distress (Wilkes, Gledhill and Smyth, 1983, Goldfinch, McNaughton and Davies, 1988), particularly those exceeding 300 $mg \cdot kg^{-1}$ body mass as proposed by McNaughton (McNaughton, 1992), smaller doses may minimise the risk of side-effects. In contrast, incidences of stomach cramp, bowel urgency and diarrhoea have still been reported using a dose ≤ 300 $mg \cdot kg^{-1}$ body mass (Miller et al. 2016), highlighting the need for using an individualised approach. Athletes are therefore encouraged to pilot $NaHCO_3$ supplementation pre-season or during training to establish their tolerance to various doses.

2.6.3 Gastro-resistant formulations

Cellulose is a polysaccharide consisting of a linear chain of glucose (Figure 2.3) and is present in plant cell walls (Barbosa, Conway and Merchant, 2017). Given that cellulose is resistant to acidic pH and becomes soluble in less acidic conditions, cellulose-based capsules have been designed to withstand the acidic environment of the stomach (i.e. enteric-formulated).

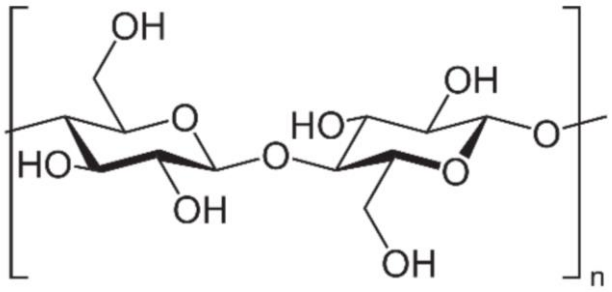

Figure 2.3 The chemical structure of cellulose (Barbosa, Conway and Merchant, 2017).

Using baths to simulate the gastric and intestinal environments of humans, gastro-resistant capsules resist degradation at high acidity (pH 1-2 AU) and typically degrade in the more alkaline environment (pH 6-7 AU). Preventing capsule degradation can therefore postpone the release of $NaHCO_3$, which in turn, prevents gastric acid from being neutralised and reduces symptoms such as nausea and vomiting (Barbosa, Conway and Merchant, 2017). Whereas enteric-coated capsules are suggested to bypass the stomach entirely, the gastro-resistant functionality of delayed-release capsules is variable. Furthermore, it is generally accepted that enteric-coated capsules breakdown further into the GI tract and are considered more gastro-resistant compared with delayed-release capsules.

2.7 Summary

In summary, GI side-effects appear to be associated with $NaHCO_3$ supplementation, particularly following the oral ingestion of ergogenic doses. While several mechanisms have been proposed, the neutralisation of gastric acid is thought to be a contributing factor. Nevertheless, little research has attempted to reduce the prevalence and severity of GI symptoms, and athletes currently have limited practical options to minimise these adverse effects. However, there is a growing research interest in nutritional strategies that may optimise $NaHCO_3$ supplementation using novel ingestion forms. Fundamentally, there is a need to resolve the challenges inherent with using this nutritional supplement, focusing on alternatives that attenuate the side-effects but still offer a performance-enhancing effect.

Chapter 3: General Methods

The purpose of this chapter is to outline the procedures that will remain consistent across all studies that compose this thesis. This will include a description, justification and, were applicable, the test-retest reliability data. Experimental design and methods that are specific to each study will be described in the relevant chapters.

3.1 Ethical considerations

Ethical approval was granted for all studies in this thesis from the Department of Sport and Physical Activity Research Ethics Committee and the University Research Ethics Sub-Committee at Edge Hill University. Blood sampling was carried out in accordance to the local Human Tissue Authority license regulations and no blood samples were stored. Participation in the study was entirely voluntary and no remuneration was provided. All participants were given a Participant Information Sheet that clearly outlined study commitments, experimental procedures and any associated risks. Participants were given at least one week to read the document and fully consider the requirements of participation. Furthermore, study protocols were explained in full and questions were answered prior to gaining written informed consent to participate. Participants had the right to withdraw from the study during experimentation and up until the point of individual data collection being completed. No participants requested to withdraw their consent during any study. All testing was carried out at the Physiology laboratory in the Department of Sport and Physical Activity, Edge Hill University.

All participants were required to complete a medical screening questionnaire before taking part in any study to minimise the risk of adverse events during exercise. Participants were also asked to inform the researcher if there was a change in health status throughout experimental testing. Participants also completed a pre-exercise screening questionnaire before each exercise trial (Chapter 6). This also involved measuring heart rate and blood pressure. Heart rate was measured using manual palpation for one minute, whereas blood pressure was

measured using an automated sphygmomanometer. Inclusion criteria was systolic blood pressure ≤ 140 mmHg, diastolic blood pressure ≤ 90 mmHg and heart rate < 90 beats·min^{-1} (and regular on palpation). If any measure exceeded this criteria exercise was not be permitted and the participant was advised to seek medical advice before being reconsidered.

3.2 Participants

Healthy adult males between the ages of 18-60 years were recruited to participate in each study. None of the participants had a history of GI disease and none were under pharmacological intervention during any study. Exclusion criteria included those with hypertension, renal impairment or following a salt-restricted diet. Specific participant characteristics are described in each study chapter. Training status was classified in accordance with the criteria outlined by De Pauw et al. (2013). In Chapters 4 and 5, participants are described as recreationally trained due to participating in regular exercise (> 4 h·week^{-1}) and having a peak oxygen uptake ($\dot{V}O_{2peak}$) between 45.0-54.9 ml·kg^{-1}·min^{-1}. In Chapter 6, the participants are described as trained cyclists based on meeting the following criteria; $\dot{V}O_{2peak}$ 55.0-64.9 ml·kg^{-1}·min^{-1}, training frequency ≥ 3 d·week^{-1} and cycling distance > 60 km·week^{-1}.

3.3 Experimental procedures

Before each experimental trial, participants undertook several standardised procedures to limit the confounding effects of nutrition, hydration status and day-to-day physiological variation. These procedures were confirmed verbally on arrival to the laboratory during each visit. Experimental trials for example, were conducted at the same time of day (0900 h) to minimise the physiological effects of circadian rhythms (Reilly, 1990). Participants were asked to maintain habitual physical activity levels during each study and abstain from alcohol and

caffeine consumption for 12 h, and strenuous exercise 24 h before each laboratory visit (Weststrate et al. 1990, Westerterp-Plantenga et al. 2006). Water intake was encouraged in the 24 h preceding experimental testing sessions to ensure euhydration. A normal habitual diet was maintained, although participants attended the laboratory following an overnight fast (~ 12 h). All experimental trials took place under ambient laboratory conditions (room temperature = 18-20 °C, humidity = 40-50%).

3.4 Determination of peak oxygen uptake

During the experimental studies, $\dot{V}O_{2peak}$ was determined using an incremental exercise test to volitional exhaustion on an electromagnetically-braked cycle ergometer (Lode Excalibur Sport, Groningen, The Netherlands). After a 5 min warm-up at 70 watts (W), the workload increased by 30 $W \cdot min^{-1}$ (1 W every 2 s) until volitional exhaustion. Participants selected a preferred cadence (70–120 $rev \cdot min^{-1}$) to maintain throughout, with volitional exhaustion defined as an inability to maintain > 60% of this cadence for > 5 s despite strong verbal encouragement. Breath-by-breath gases were used to measure oxygen uptake ($\dot{V}O_2$), carbon dioxide production ($\dot{V}CO_2$), minute ventilation ($\dot{V}E$) and the respiratory exchange ratio (RER). Heart rate (Polar®, Kempele, Finland) and rating of perceived exertion (RPE) were also recorded each minute (Borg, 1973). The following criterion were used to confirm that $\dot{V}O_{2peak}$ had been reached: (i) heart rate within 10 $beats \cdot min^{-1}$ of age-predicted maximum; (ii) respiratory exchange ratio > 1.10 arbitrary units (AU); (iii) RPE > 18/20 AU (Midgley et al. 2007). Data were then averaged into 10 s bins with $\dot{V}O_{2peak}$ defined as the highest 10 s average of oxygen uptake ($\dot{V}O_2$) obtained during the test.

3.5 Gas analysis

Breath-by-breath gases were measured continuously during exercise using an online gas analysis system (Oxycon Pro™, Jaeger, Germany). The Oxycon Pro™ has previously been reported to be both valid and reliable, as it possesses a relatively low (< 7%) coefficient of variation (CV) for all parameters (Carter and Jeukendrup, 2002). Prior to each use, the gas analysis system was switched on after a warm-up period (1 h) was allowed, before being calibrated in accordance with manufacturer instructions. This involved using an automated calibration procedure using a calibration gas (16.25% oxygen, 4.13% carbon dioxide and 79.62% nitrogen), which was repeated until the current data for gain, offset and delay time were within very low (≤1%). Furthermore, volume calibration was conducted using a 3 L syringe (Hans Rudolph, USA), whereby six complete pumps of the syringe were performed until the difference between the current and previous volume calibration was very low (≤1%). As data were collected continuously during exercise, participants were required to wear a face mask (Hans Rudolph, USA).

3.6 Heart rate

Heart rate will be recorded continuously during exercise using a telemetric monitoring system (Polar®, Kempele, Finland). This required participants to wear a heart rate transmitter belt across their chest in line with the xiphoid process and with the electrodes moistened to enhance signal amplitude. Heart rate was recorded at several time points during each trial, which are described in the relevant study chapters.

3.7 Blood sampling

Fingertip capillary blood samples (95 μL) were taken at various time points, which are described in each study chapter. All samples were taken using a disposable lancet (AccuCheck Safe-T-Pro, Indianapolis, USA) using an aseptic technique and collected in heparin-coated glass capillary tubes (Radiometer Medical Ltd, Denmark). Samples were analysed immediately (Radiometer ABL800 BASIC, Denmark) for acid-base balance and electrolyte content, including blood [HCO_3^-] and pH, as well as blood sodium ([Na^+]), potassium ([K^+]) and chloride ([Cl^-]) ion concentrations. The ABL800 BASIC radiometer has been shown good to excellent criterion validity compared with other commercially available devices (Stadlbauer et al. 2011). Furthermore, good to excellent test-retest reliability has been shown for blood [HCO_3^-] (r = 0.76-0.94; CV < 5%) and pH (r = 0.62-0.71; CV < 5%) using a 300 $mg \cdot kg^{-1}$ body mass dose (Gough et al. 2017b). The ABL800 BASIC radiometer in the Physiology laboratory at Edge Hill University has also demonstrated excellent test-retest reliability (CV < 5%) in a small pilot study. During exercise trials, further 5 μL blood samples were collected to measure blood lactate ion concentration ([La^-]) at various intervals. Samples were measured using a portable analysing device (Lactate Pro 2, LT-1730, Arkray, Japan) that has demonstrated good reliability (Pyne et al. 2000). Blood bicarbonate kinetics were used to determine characteristics of the ingestion forms, including lag time (T_{lag}), peak blood [HCO_3^-] (C_{max}), change in C_{max} (ΔC_{max}), time-to-reach C_{max} (T_{max}) and area under the curve (AUC). Bicarbonate T_{lag} was defined as the point at which blood [HCO_3^-] increased beyond normal daily fluctuation.

3.8 Perceptual measures

Throughout the exercise trials, perceived exertion and perceived fatigue were measured at regular intervals, as previously suggested (Micklewright et al. 2017). Lower-limb ratings of

perceived exertion (RPE-L) and RPE were recorded using a 6–20 scale (Borg, 1982), whereas perceived ratings of fatigue (ROF) were recorded on a 10-point Likert scale (Micklewright et al. 2017). Symptoms of GI distress were recorded using an adapted GI symptom questionnaire (Carr et al. 2011) including nausea, flatulence, stomach cramping, belching, stomach ache, bowel urgency, diarrhoea, vomiting, and stomach bloating. Symptoms were self-measured on a 10 cm visual analogue scale where "0 = No symptom" and "10 = Severe symptom" (Miller et al. 2016). Symptom terminology was explained to participants before the experimental trials commenced to ensure consistency in the reporting of symptoms.

Chapter 4: The Effects of Delayed-Release Sodium Bicarbonate Supplementation on Gastrointestinal Symptoms and Markers of Acid-Base Balance

4.1 Introduction

Acute bicarbonate loading with $NaHCO_3$ is a well-established nutritional ergogenic strategy. Supplementation can improve short-duration (1-10 min), high-intensity exercise performance (McNaughton et al. 2016), with various meta-analyses confirming its efficacy (Matson and Tran, 1993, Carr, Hopkins and Gore, 2011, Christensen et al. 2017). As an extracellular buffering agent, $NaHCO_3$ enhances endogenous bicarbonate buffering capacity by inducing significant, albeit transient, elevations in extracellular bicarbonate. Consequently, this enhances efflux of H^+ from skeletal muscle, therefore delaying muscle fatigue and positively affecting numerous performance variables, such as power output (Bellinger et al. 2012) and time to exhaustion (Higgins, James and Price, 2013). Whilst it remains unclear whether minimal increases are required to achieve these benefits, substantial changes (~6 $mmol \cdot L^{-1}$) in [HCO_3^-] may improve the likelihood of performance enhancing effects (Matson and Tran, 1993, Carr, Hopkins and Gore, 2011, Bellinger et al. 2012, Higgins, James and Price, 2013, Christensen et al. 2017, Heibel et al. 2018). Given that bicarbonate is lost in the neutralisation of gastric acid (Turnberg et al. 1970), large oral doses (200-300 $mg \cdot kg^{-1}$ body mass) are required to induce meaningful elevations in the blood.

Acute GI distress is a known side-effect of ingesting large amounts of $NaHCO_3$ (Burke and Pyne, 2007), particularly when administered as a solution (Carr et al. 2011). Ergogenic effects have still been observed in those reporting GI distress (McNaughton et al. 2016, Miller et al. 2016), however there is evidence to suggest that GI distress may be ergolytic for some individuals (Kahle et al. 2013, Saunders et al. 2014a, McNaughton et al. 2016, Barbosa, Conway and Merchant, 2017). Furthermore, some authors have suggested that GI distress may deter individuals from using $NaHCO_3$ regardless of its potential ergogenic benefits (Heibel et al. 2018). Although the impact of GI distress on performance remains ambiguous, symptoms, including vomiting and diarrhoea, may present a major practical limitation for athletes and

coaches. Whilst split-dose protocols can help to alleviate GI symptoms (Sale et al. 2011, Saunders et al. 2014a, Saunders et al. 2014b), there is little consensus regarding how this protocol should be implemented, and the evidence relating to exercise performance is scarce. Furthermore, most research studies have adopted an acute loading protocol which is ingested 60-90 min prior to exercise (Renfree, 2007, Price and Simons, 2010, Carr et al. 2011, Siegler et al. 2012). Developing novel acute loading strategies which can alleviate GI symptoms may therefore represent a more practical ingestion strategy for athletes during training and/or competition.

Gastro-resistant capsules can resist gastric degradation and reduce GI symptoms provoked by acid sensitive compounds, such as $NaHCO_3$ (Farias de Oliveira, Saunders and Artioli, 2018). Hydroxypropyl methylcellulose, contained in delayed-release capsules, can resist degradation in acidic environments (pH ~1-2 arbitrary units (AU)), and therefore provides gastro-resistant properties. Instead, degradation occurs in the duodenum were the pH is far more alkaline (pH ~ 6-7 AU) and absorption can take place rapidly. Since GI distress is partly attributable to degradation in the stomach (Turnberg et al. 1970), it has been suggested that gastro-resistant capsules may alleviate symptoms that are typical with $NaHCO_3$ ingestion (Farias de Oliveira, Saunders and Artioli, 2018). Given that less bicarbonate is lost in the stomach, it has also been suggested that smaller doses may produce comparable acid-base changes to larger doses (Farias de Oliveira, Saunders and Artioli, 2018). In contrast, as the time available for absorption is reduced with gastro-resistant formulations (Barbosa, Conway and Merchant, 2017), this may reduce bicarbonate bioavailability when administered in this form. No study to date has examined the use of delayed-release $NaHCO_3$ on markers of GI distress, nor on bicarbonate bioavailability and subsequent blood acid-base responses. Reducing GI distress following $NaHCO_3$ ingestion may enhance use by athletes, particularly among those who are deterred by potential side-effects.

The aim of this study was, therefore, to investigate whether delayed-release $NaHCO_3$ could mitigate GI distress compared with a solution using an acute loading protocol, as well as compare the acid-base responses following supplementation.

4.2 Methods

4.2.1 Participants

Twelve trained (according to DePauw et al. 2013) healthy males (age 26 ± 5 y, $\dot{V}O_{2peak}$ 58.9 ± 10.9 ml·kg^{-1}·min^{-1}, height 1.8 ± 0.1 m, body mass 82.3 ± 11.1 kg, fat-free mass 72.3 ± 10.0 kg) were recruited for the study. The study was approved by the University Research Ethics Committee before the participants gave written informed consent to take part in the study. Inclusion in the study required that participants had performed regular (≥ 3 d·week^{-1}) physical exercise for at least two years. Exclusion criteria included ingestion of any buffering agents < 6 months prior to commencing the study, and those with hypertension or on a sodium-restricted diet.

4.2.2 Study overview

Before taking part in the experimental trials, each participant underwent a baseline assessment over two laboratory visits separated by at least 48 h to establish (i) body composition and $\dot{V}O_{2peak}$ and (ii) fluctuations in blood analytes ([HCO_3^-] and pH) under normal conditions. Fluctuations in blood analytes and GI symptoms under normal conditions were used as a control measure throughout. In the experimental trials, all participants underwent two conditions; 300 mg·kg^{-1} body mass $NaHCO_3$ administered as either a solution or encased in delayed-release capsules. Experimental trials were administered in a block randomised crossover design that were counterbalanced (Latin-square) in order of administration, and took place at least seven days apart to allow for the washout of residual $NaHCO_3$ (Bishop et al.

2004). Participants were required to abstain from alcohol or caffeine-containing beverages for 12 h and strenuous exercise 24 h before each laboratory visit. All sessions took place under standardised laboratory conditions (temperature = 21–22 °C, relative humidity = 50–55%, barometric pressure = 756–759 mmHg) and were conducted at 0900 h to account for circadian rhythms (Reilly, 1990).

4.2.3 Baseline assessment

Participants arrived at the laboratory on both occasions after an overnight fast (~12 h) and euhydrated. On one visit, semi-nude body mass and fat-free mass were assessed using whole-body air displacement plethysmography (BOD POD®, COSMED, Italy). Participants then performed an incremental exercise test to volitional exhaustion on an electromagnetically-braked cycle ergometer (Excalibur Sport, Lode, Netherlands). After a standardised 5 min warm-up at a power output of 70 W, the cycling protocol commenced at 75 W for 1 min and workload increased by 1 W every 2 s (30 $W \cdot min^{-1}$) until volitional exhaustion. This was determined by the inability of the participant to sustain their respective self-selected cadence for > 5 s despite feedback and strong verbal encouragement. On a separate visit, fingertip capillary blood samples were obtained using an aseptic technique after the participants were quietly seated for 20 min. Blood samples were drawn every 20 min over 180 min, with 10 min sampling between 80 and 140 min to accurately capture peak values (Gough et al. 2017b). Blood samples were collected in 100 μL heparin-coated glass capillary tubes (Radiometer Medical Ltd, Denmark) and immediately analysed using a blood gas analyser (ABL800 BASIC, Radiometer Medical Ltd., Denmark). At the same time points, GI symptoms were recorded using a 9-item questionnaire, including nausea, flatulence, stomach cramping, belching, stomach ache, bowel urgency, diarrhoea, vomiting and stomach bloating (Carr et al. 2011). Symptoms were self-measured on a 10 cm scale, the ends of which were marked “0, no

symptom" and "10, severe symptom", as previously described (Miller et al. 2016). Participants remained seated throughout, although toilet breaks were permitted. No food was consumed during the experimental trials and water was permitted *ad libitum*, with volumes replicated in the subsequent experimental session.

4.2.4 Experimental trials

Solutions were prepared in 400 ml of natural mineral water (Evian®, France) and mixed with 50 ml of sugar-free blackcurrant flavoured squash (Robinsons®, UK) and refrigerated (~1 h) to enhance palatability (Miller et al. 2016) . For the delayed-release condition, size 00 capsules (DRcaps™, Capsugel®, France) were prefilled with $NaHCO_3$ using a capsule filling device (Capsule Connection LLC, USA), while doses were checked for accuracy using digital laboratory scales (Fisher, OHAUS™). Participants were instructed to ingest either the solution or delayed-release capsules with an equal volume (400 ml) of water within 10 min, while the stopwatch commenced parallel with the start of ingestion (Jones et al. 2016, Gough et al. 2017b). All experimental trials were conducted under the same conditions as the control trial, with blood analytes and GI symptoms measured as previously described.

4.2.5 Statistical analyses

Prospective statistical power analysis was conducted *a priori* to determine that twelve participants were required, with alpha and beta set at 0.05 and 0.20, respectively. Data were assessed for normality using standard graphical methods prior to analyses (Grafen and Hails, 2002). Two-way analysis of variance (ANOVA) with repeated measures (condition × time) was used to establish significant main effects for blood analytes ($[HCO_3^-]$, pH and BE) and GI symptom scores. Condition consisted of two levels (solution and delayed-release capsules) whereas time consisted of thirteen (0, 20, 40, 60, 80, 90, 100, 110, 120, 130 140, 160 and 180

min). Effect sizes were calculated using partial eta-squared (ηp^2) for ANOVA and were interpreted according to Cohen (Cohen, 1988) as follows: trivial <0.20; small 0.20-0.49; moderate 0.50-0.79 and large ≥ 0.80. Sphericity was assessed using Mauchly's test throughout. Where appropriate, corrections for violations of sphericity (Greenhouse-Geisser) and multiple comparisons of differences within a factor (Bonferroni) were made (Atkinson, 2002). Mean HCO_3^- kinetic variables and highest GI symptoms score between conditions were analysed by paired samples *t*-test. Descriptive data are presented as either mean ± standard deviation (SD) unless stated otherwise. The α-level of statistical significance was set at $P < 0.05$, and exact *P*-values are given in the text and tables. Values for *P* of "0.000" given by the statistical package were corrected to "< 0.0005" (Kinnear and Gray, 1995). Data were analysed using the Statistical Package for the Social Sciences (SPSS®) for Windows® (IBM, Chicago, IL, USA), version 25.

4.3 Results

4.3.1 Gastrointestinal symptoms

Frequency

No GI symptoms were reported pre-ingestion, nor at any time point in the control condition. All participants (n = 12) experienced at least one GI symptom following either solution or capsule ingestion (Table 4.1). Stomach bloating was the most prevalent GI symptom in both experimental trials, although the prevalence was lower with delayed-release capsules (58%) than with the solution (100%). Overall, fewer GI symptoms (mean difference −45.1%) were reported with delayed-release capsules than with a solution (Figure 4.1). Furthermore, each GI symptom was less frequent with delayed-release capsules than with the solution (Figure 4.2).

Table 4.1. The most severe individual GI symptom reported during any trial. Symptom scores are displayed in parenthesis and are expressed as arbitrary units (AU).

Participant	Control	Solution	Delayed-release
1	No symptom (0.0)	Stomach cramp (3.5)	Stomach bloating (3.0)
2	No symptom (0.0)	Bowl urgency (7.0)	Stomach bloating (3.0)
3	No symptom (0.0)	Nausea (6.0)	Nausea (2.0)
4	No symptom (0.0)	Diarrhoea (10.0)	Stomach bloating (7.0)
5	No symptom (0.0)	Diarrhoea (10.0)	Diarrhoea (7.0)
6	No symptom (0.0)	Diarrhoea (10.0)	Diarrhoea (5.5)
7	No symptom (0.0)	Bowl urgency (6.0)	Bowl urgency (5.0)
8	No symptom (0.0)	Bowl urgency (10.0)	Bowl urgency (2.0)
9	No symptom (0.0)	Diarrhoea (10.0)	Belching (3.0)
10	No symptom (0.0)	Diarrhoea (10.0)	Belching (3.0)
11	No symptom (0.0)	Stomach ache (3.0)	Belching (3.0)
12	No symptom (0.0)	Diarrhoea (10.0)	Diarrhoea (7.0)
Mean (SD)	0.0 ± 0.0 AU	8.0 ± 2.7 AU	4.2 ± 2.0 AU

Severity

Symptom severity increased in the solution ($P < 0.0005$) and delayed-release ($P = 0.017$) conditions beyond those shown with the control. There was a significant effect of ingestion form ($F_{1.00, 11.00} = 21.13$, $P = 0.001$, $\eta p^2 = 0.66$), with less severe GI symptoms reported with

delayed-release capsules than with the solution ($P = 0.001$; Figure 4.1). There was no effect of time ($F_{2.85, 31.36} = 2.89$, $P = 0.053$, $\eta p^2 = 0.21$) and no significant condition × time interaction was found ($F_{3.22, 35.39} = 1.87$, $P = 0.148$, $\eta p^2 = 0.15$). The most severe GI symptom reported with delayed-release capsules (4.2 ± 2.0 AU) was significantly lower ($P = 0.002$) than with the solution (8.0 ± 2.7 AU).

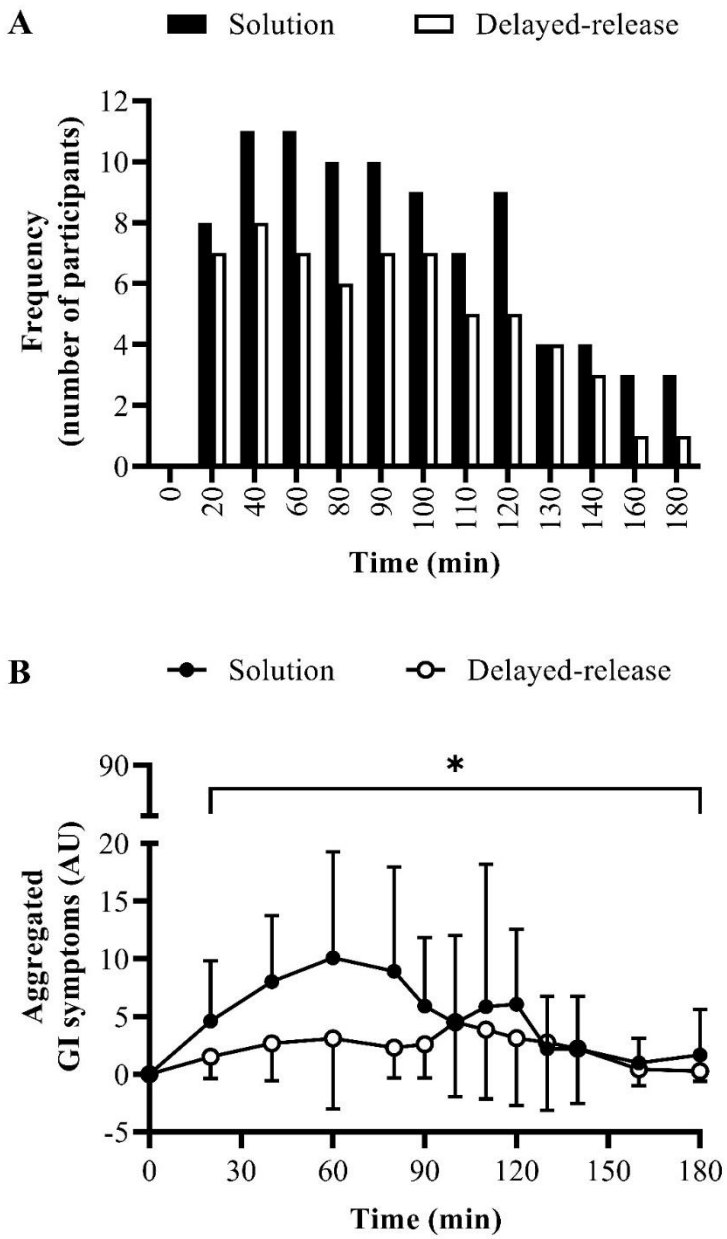

Figure 4.1. Incidence (A) and mean (± SD) severity (B) of GI symptoms. *Denotes significant difference between conditions ($P < 0.05$).

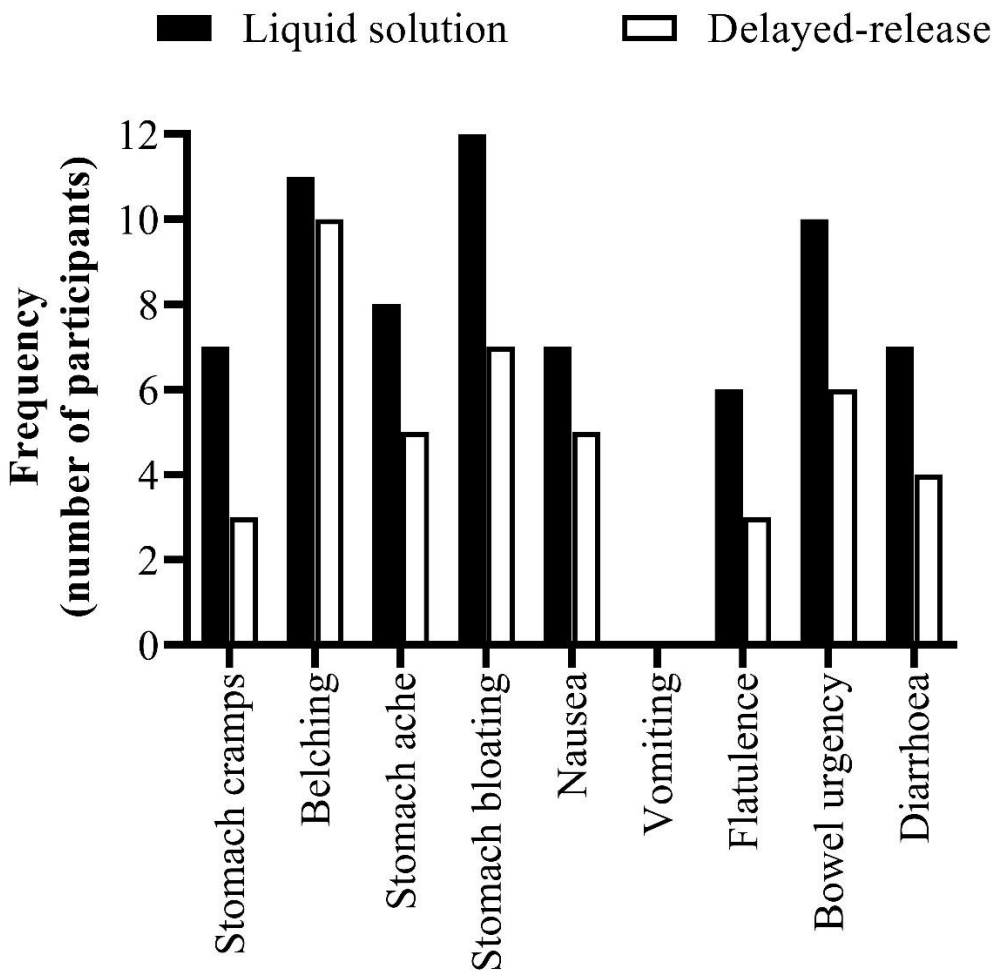

Figure 4.2. Frequency of each GI symptom reported by all participants (n = 12). Values represent the number of participants that reported each symptom.

4.3.2 Acid-base balance

Bicarbonate

Ingestion form had no significant effect on blood [HCO_3^-] ($F_{1.00, 11.00} = 0.71$, $P > 0.05$, $\eta p^2 = 0.061$) up to 180 min post-ingestion. There was a significant effect of time ($F_{2.38, 26.23} = 101.74$, $P < 0.0005$, $\eta p^2 = 0.90$); blood [HCO_3^-] increased from the previous time point for 60 min post-ingestion ($P < 0.05$), with no significant change up to 180 min post-ingestion ($P > 0.005$, Figure 4.3). A significant interaction was found between condition and time ($F_{2.31, 25.44} = 16.48$,

$P < 0.0005$, $\eta p^2 = 0.60$). Blood [HCO_3^-] were significantly higher with the solution at 20 ($P = 0.008$), 40 ($P = 0.001$) and 60 min ($P = 0.011$) than with the delayed-release capsules, and significantly lower at 130 ($P = 0.021$), 140 ($P = 0.019$) and 160 min ($P = 0.047$).

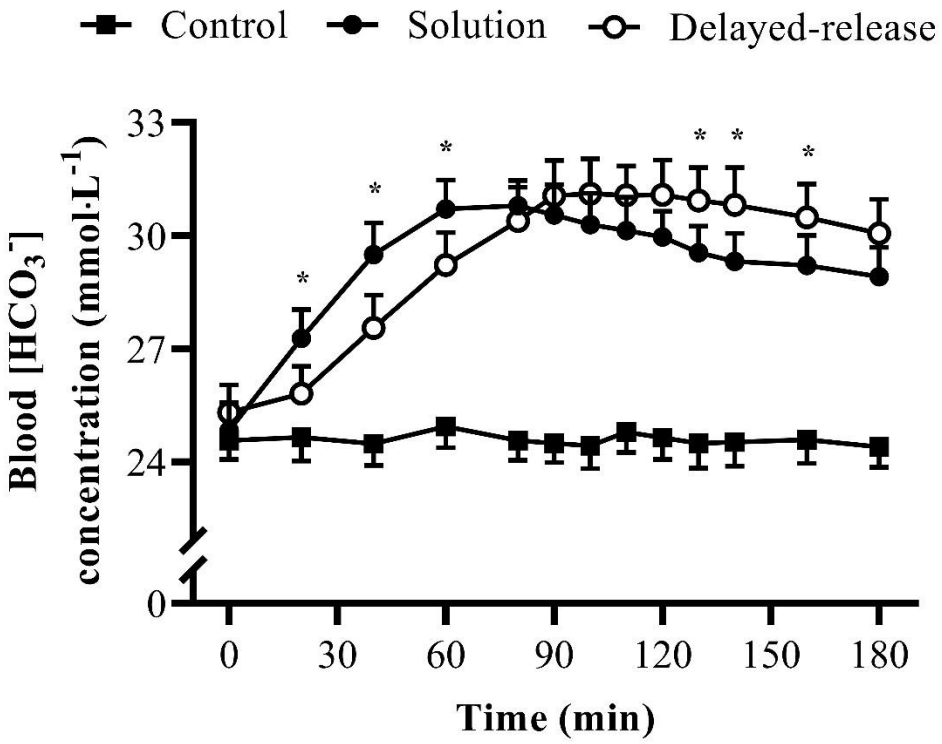

Figure 4.3. Mean (± 95% confidence interval [CI]) blood [HCO_3^-]. *Denotes significant difference between solution and delayed-release capsules ($P < 0.05$).

Mean bicarbonate kinetic variables are displayed in Table 4.2. More participants reached a 5 mmol·L^{-1} (solution: n = 10; delayed-release: n = 11) and 6 mmol·L^{-1} (solution: n = 8; delayed-release: n = 9) increase in blood [HCO_3^-]with delayed-release capsules than with the solution (Figure 4.4).

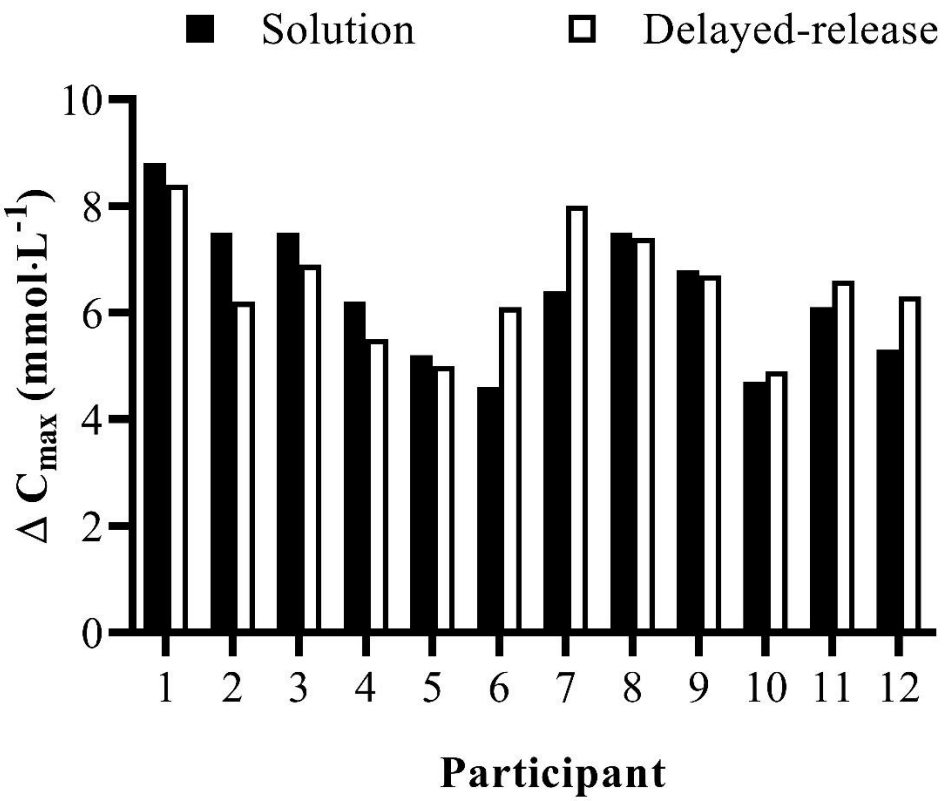

Figure 4.4. Individual changes in blood [HCO_3^-] between solution and delayed-release capsules.

pH

Ingestion form had no significant effect on pH ($F_{1.00, 11.00} = 2.88$, $P = 0.188$, $\eta p^2 = 0.21$) up to 180 min post-ingestion. There was a significant effect of time ($F_{4.42, 48.60} = 43.74$, $P < 0.0005$, $\eta p^2 = 0.80$); blood pH increased markedly for 60 min ($P < 0.05$) post-ingestion, until a decrease occurred from the previous time point at 70 min ($P < 0.0005$) and continued to decrease up to 180 min ($P < 0.05$) post-ingestion (Figure 4.5). A significant interaction was found between condition and time for pH ($F_{4.88, 53.67} = 6.42$, $P < 0.0005$, $\eta p^2 = 0.37$). Blood pH was significantly higher with the solution at 40 min ($P = 0.009$) than with the delayed-release capsules, and significantly lower at 120 min ($P = 0.017$) post-ingestion.

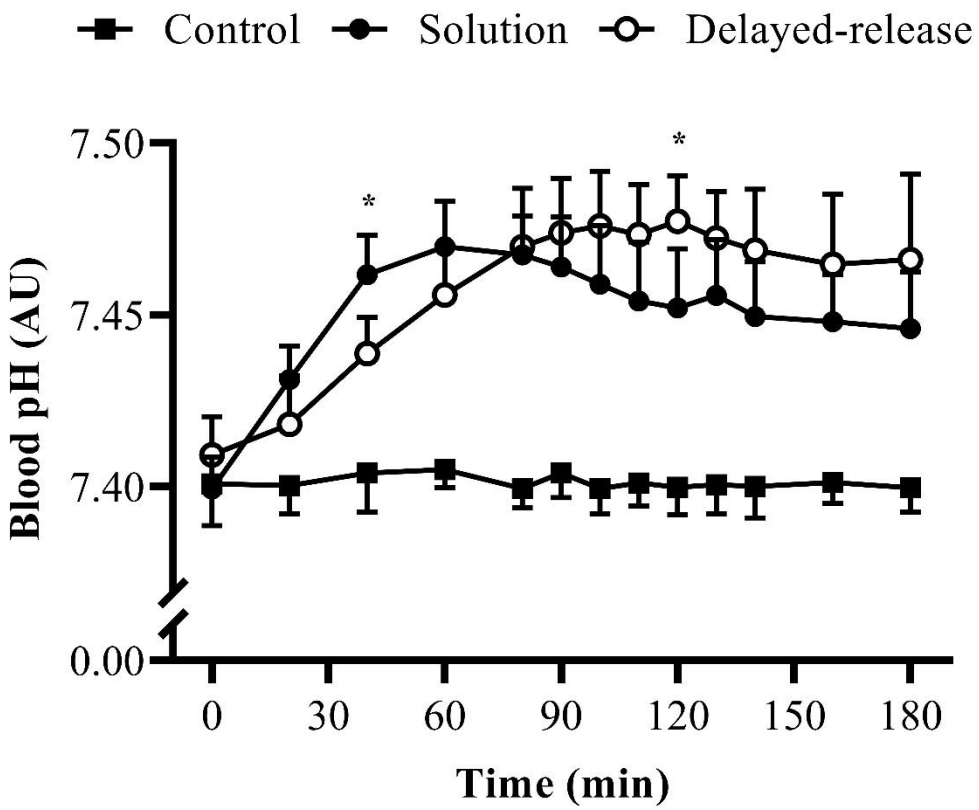

Figure 4.5. Mean (± 95% CI) blood pH responses. *Denotes significant difference between solution and delayed-release capsules ($P < 0.05$).

Blood pH peaked much later with the delayed release capsules (solution = 72 ± 26 min; delayed-release = 125 ± 28 min; $P = 0.001$) than with the solution, although absolute changes were comparable between conditions (solution = 0.08 ± 0.02 AU; delayed-release = 0.08 ± 0.02 AU; $P = 0.093$).

Table 4.2. Mean (± SD) bicarbonate kinetics in the solution and delayed-release conditions, together with the statistical significance of the difference.

Variable	**Solution**	**Delayed-release**	***t*-test**	***P* value**
T_{lag} (min)	20 ± 0*	32 ± 10 *	-3.92	**0.002**
T_{max} (min)	72 ± 18**	120 ± 29**	-5.35	**<0.0005**
C_{max} (mmol·L^{-1})	31.2 ± 1.1	31.8 ± 1.3	-1.66	0.125
Δ C_{max} (mmol·L^{-1})	6.4 ± 1.3	6.5 ± 1.1	-0.46	0.658
AUC (mmol·min/L)	5278 ± 174	5286 ± 198	-0.13	0.899

Note: Asterix denotes significant difference between conditons (*$P < 0.05$; **$P < 0.0005$). T_{lag}, time to commence change in blood bicarbonate ion concentration; T_{max}, time to reach peak blood bicarbonate ion concentration; C_{max}, peak blood bicarbonate ion concentration; Δ C_{max}, absolute change in blood bicarbonate ion concentration; AUC, area under the concentration-time curve.

4.4 Discussion

This is the first study to investigate the effects of gastro-resistant capsules on GI distress, bicarbonate bioavailability and subsequent acid-base responses following $NaHCO_3$ ingestion. The main finding was that delayed-release $NaHCO_3$ mitigated GI distress. Fewer GI symptoms were reported with the delayed-release capsules, and the overall severity was reduced when compared to the solution; a finding that has been suggested in the relevant literature (Farias de Oliveira, Saunders and Artioli, 2018). Gastrointestinal symptoms were negated with the delayed-release capsules, with a reduction in the most severe symptom experienced up to 3 h

following $NaHCO_3$ supplementation (Table 4.1). Given that GI symptoms may be ergolytic (McNaughton, 1992, Kahle et al. 2013, Saunders et al. 2014a), delayed-release $NaHCO_3$ may be more ergogenic in those who experience GI distress with the solution. Furthermore, since GI distress may deter some individuals from using $NaHCO_3$ as an ergogenic aid (Carr et al. 2011, Heibel et al. 2018), delayed-release $NaHCO_3$ would appear to be a more favourable option for athletes and coaches.

While necessary to achieve erogenicity (Jones et al. 2016), large boluses (200-300 $mg \cdot kg^{-1}$ body mass) of $NaHCO_3$ can induce significant GI symptoms. In the current study, there was a high incidence of GI distress with the solution, which is in agreement with some authors (Kahle et al. 2013) but not others (Van Montfoort et al. 2004, Carr et al. 2011). On entering the stomach, $NaHCO_3$ dissociates to sodium and [HCO_3^-], the latter of which produces carbon dioxide during the neutralisation of gastric acid (Turnberg et al. 1970). Consequently, carbon dioxide tension increases exponentially with exposure and is associated with symptoms, such as belching, nausea and stomach ache. Delayed-release capsules, partly formulated with an enteric barrier, display gastro-resistant properties and can minimise disintegration in the stomach. Mitigating GI symptoms may indeed have implications for performance. Previous research indicates that symptoms can inhibit high-intensity cycling performance (Saunders et al. 2014a), while others have reported improvements irrespectively (McNaughton, 1992). Since numerous participants have withdrawn from studies due to GI distress (Gough et al. 2017a), previous research may have underestimated the ergolytic effect of such symptoms. Studies that have attempted to mitigate GI symptoms following $NaHCO_3$ ingestion have used alternative dosing strategies. Gelatine capsules co-ingested with a small high-carbohydrate (1.5 $g \cdot kg^{-1}$ body mass) meal is currently regarded as the formulation least likely to induce GI symptoms following $NaHCO_3$ ingestion (Carr et al. 2011). In the current study, delayed-release capsules were ingested after an overnight fast, largely to minimise potential confounding effects of food

on acid-base changes. Nevertheless, co-ingestion with a small high-carbohydrate meal may have further reduced GI symptoms and warrants further investigation. Furthermore, while comparison with a solution was chosen based on its frequency of use within the literature, this may not be the case in the practice and is thus a limitation to the study. Further research should therefore look to assess potential differences in GI and acid-base responses following $NaHCO_3$ supplementation using different capsules.

In relation to bioavailability, both ingestion forms provided adequate sources of [HCO_3^-] and displayed similar kinetics. Increases in [HCO_3^-] were comparable, with both forms exceeding the 6 mmol·L^{-1} threshold suggested to enhance ergogenicity (Carr, Hopkins and Gore, 2011). Interestingly, some participants displayed enhanced bicarbonate availability (≥ 1 mmol·L^{-1}) with delayed-release capsules (Figure 4.4), while only one participant was found to have enhanced bicarbonate availability of this magnitude with the solution. Similarly, more participants achieved a 5 to 6 mmol·L^{-1} increase in [HCO_3^-] with the delayed-release capsules than with the solution. These results would be explained by the gastric bypass model proposed by Oliveira et al (Farias de Oliveira, Saunders and Artioli, 2018), which includes the effect of gastric transit time and HCO_3^- loss associated with neutralisation. As suggested by these authors, reducing HCO_3^- neutralisation in the stomach increases bioavailability when $NaHCO_3$ is administered orally. Since changes of ~ 1 mmol·L^{-1} in blood [HCO_3^-] can positively affect performance (McNaughton, 1992), delayed-release $NaHCO_3$ may be more ergogenic. In contrast to the solution, [HCO_3^-] absorption did not commence immediately following capsule ingestion, suggesting that the delayed-release capsules were effective (Marzorati et al. 2015, Barbosa, Conway and Merchant, 2017). This result indicates that the capsules achieved disintegration in the intestine, which had the effect of lengthening (+ 48 min) the time to reach peak [HCO_3^-]. Blood [HCO_3^-] peaked at ~ 120 min post-ingestion with the delayed-release capsules, which is later than previously reported with a solution in some studies (Gough et al.

2017b) but not all (Jones et al. 2016). Similar to previous studies (Renfree, 2007, Siegler et al. 2010, 2012), there was a high degree of individual variability in the time to reach peak [HCO_3^-], although this was greater with the capsules. When deciding an appropriate ingestion form, it is therefore advisable that athletes continue to monitor individual concentration-time profiles following $NaHCO_3$ supplementation.

Metabolic alkalosis was induced earlier with the solution (~ 40 min) than with delayed-release capsules (~ 60 min), although this state was maintained for longer (+ 30 min) when ingested in the delayed-release form. Exercise performance timed with peak alkalosis may enhance the ergogenicity of $NaHCO_3$ supplementation (Gough et al. 2017a, Heibel et al. 2018), therefore it is reasonable to consider that delayed-release may provide a larger ergogenic window. In a competitive setting, this may be more favourable since it is difficult to time exercise performance peak alkalosis due to variable factors, such as sports fixtures. In the current study, time to reach peak pH was much later with delayed-release $NaHCO_3$ than with the solution. In a practical setting, delayed-release $NaHCO_3$ would have to be ingested sooner than a solution to elicit similar acid-base changes (i.e. 90-120 min before exercise).

4.5 Conclusion

In summary, delayed-release $NaHCO_3$ mitigates GI symptoms compared with a solution, which may be more appropriate for athletes who report symptoms post-ingestion. Given that blood [HCO_3^-] and pH peaked much later with delayed-release NaHCO3, this would have to be ingested earlier (i.e. 90-120 min before exercise) than with a solution to induce comparable acid-base changes. The current study supports the gastric bypass model, which can be used as a model for minimising GI symptoms following $NaHCO_3$ supplementation.

Chapter 5: Enteric-Coated Sodium Bicarbonate Attenuates Gastrointestinal Side-Effects and Alters Acid-Base Responses

5.1 Introduction

Acute GI disturbances are problematic following $NaHCO_3$ supplementation (Burke and Pyne, 2007, Cameron et al. 2010, Kahle et al. 2013) as shown in Study 1 (Chapter 4). Although the aetiology of GI symptoms involves multiple mechanisms, the neutralisation of gastric acid is considered a prominent factor (Turnberg et al. 1970). As shown in Study 1 (Chapter 4), gastro-resistant capsules can lesson GI symptoms compared with drink solutions, while inducing comparable acid-base balance. There is however speculation as to how the site of disintegration may alter the bioavailability of bicarbonate, which is thought to be a factor modulating its ergogenic effects (Heibel et al. 2018).

Whilst minimising the loss of bicarbonate anions in the stomach may augment blood [HCO_3^-] (Farias de Oliveira, Saunders and Artioli, 2018), delaying absorption also decreases GI transit time; which in turn may reduce changes in blood bicarbonate. These considerations suggest that bioavailability may be complex; one factor relates to GI transit time while another to the degree of neutralisation. Therefore, overall bioavailability is dependent upon on the balance between bicarbonate losses through neutralisation and GI transit time which is determined by the ingestion form. While previous research has compared the effects of administering $NaHCO_3$ in gelatine (Carr et al. 2011) and delayed-release capsules in comparison to a solution (Hilton, Leach, Sparks, et al. 2019), research has yet to compare the effects of different capsule ingestion forms. Consequently, little is currently known regarding how enteric coatings may affect GI symptoms and bicarbonate kinetics following $NaHCO_3$ supplementation. Furthermore, there is a notable lack of data regarding the efficacy of commercially available capsule formulations, despite this being a potential form of ingestion for athletes.

The primary aim of the present study was to determine the optimal ingestion form to minimise GI disturbances following $NaHCO_3$ ingestion. The secondary aim was to examine

the bioavailability of bicarbonate anions following $NaHCO_3$ ingestion across different ingestion forms.

5.2 Methods

5.2.1 Participants

Fourteen trained (DePauw et al. 2013) males (age 24 ± 5 years, body mass 80.9 ± 11.5 kg, $\dot{V}O_{2peak}$ 57.7 ± 5.3 $ml \cdot kg^{-1} \cdot min^{-1}$) volunteered for the study. All participants performed regular exercise training (≥ 3 $d \cdot week^{-1}$) for at least two years and were free of GI-related disorders. Exclusion criteria included those with hypertension, renal impairment or following a salt-restricted diet, and no participants were ingesting buffering agents or medications at the time of the study. Protocols were explained in full and questions were answered before the participants gave written, informed consent to participate in the study. Ethical approval was granted by the institutional research ethics committee.

5.2.2 Experimental overview

In a double-blind, placebo-controlled, randomised crossover design, participants attended the laboratory on five separate occasions. After an initial visit, experimental trials consisted of a placebo (PLA) and three $NaHCO_3$ trials, whereby 300 $mg \cdot kg^{-1}$ body mass $NaHCO_3$ was administered in either gelatine, delayed-release or enteric-coated capsules. According to the manufacturer, the delayed-release capsules (DRcaps™) were embedded with a polymer barrier (Hypromellose) within the shell, whereas the enteric-coated capsules (Bicarbi™) were spray-coated with the same ingredient. Experimental trials were counterbalanced (balanced Latin-square) in the order of administration and conducted at least 48 h apart to allow for blood [HCO_3^-] to return to 'normal' values. Participants were required to abstain from alcohol or

caffeine-containing beverages for 12 h, and strenuous exercise 24 h before each laboratory visit.

During the initial visit, semi-nude body mass was measured (BOD POD®, COSMED, Italy) before participants performed an incremental exercise test to volitional exhaustion on an electromagnetically-braked cycle ergometer (Excalibur Sport, Lode, Netherlands). This involved a standardised warm-up (5 min at 70 W) before the workload increased by 1 W every 2 s (30 $W \cdot min^{-1}$) until volitional exhaustion was reached.

Experimental trials took place under standardised laboratory conditions (temperature 20-22 °C, humidity 50 ± 5 %) and commenced at the same time of day to account for circadian rhythms (Reilly, 1990). Participants arrived at the laboratory after an overnight fast (~ 12 h) which was verbally confirmed upon arrival. Participants then ingested 300 $mg \cdot kg^{-1}$ body mass $NaHCO_3$ or placebo (cornflour) which were administered in size 0 opaque (white) capsules. Each experimental capsule contained 650 mg $NaHCO_3$ therefore doses were administered to the nearest whole capsule. Capsules were manually filled by the same individual using a capsule filling device (Capsule Connection LLC, USA) and checked for accuracy (Fisher, OHAUS™) before administration. Supplements were ingested with an equal volume (6 $ml \cdot kg^{-1}$ body mass) of water (Evian®, France) within 5 min while the timing commenced at the start of ingestion (Jones et al. 2016). Given that water was permitted *ad libitum* post-ingestion, volumes were recorded on the first trial and replicated thereafter. Participants remained seated throughout although toilet breaks were permitted.

5.2.3 Assessment of acid-base balance

During each trial, the exposure-response was established by determining the time course of blood [HCO_3^-] and pH. Fingertip capillary blood samples (95 μL) were obtained pre-ingestion and then every 20 min for 180 min post-ingestion, with 10 min sampling from 80 to 140 min.

Samples were collected in heparin-coated glass capillary tubes (Radiometer Medical Ltd, Denmark) using an aseptic technique and were analysed immediately (Radiometer ABL800 BASIC, Denmark) for blood [HCO_3^-] and pH.

5.2.4 Gastrointestinal symptoms

Symptoms of GI distress were recorded at the same time points using a 9-item questionnaire (Carr et al. 2011) including nausea, flatulence, stomach cramping, belching, stomach ache, bowel urgency, diarrhoea, vomiting and stomach bloating, as previously described.

5.2.5 Statistical analyses

Data were assessed for normality using standard graphical methods prior to analyses (Grafen and Hails, 2002). Blood [HCO_3^-], pH and aggregated GI symptom scores were analysed using two-way (trial × time) analysis of variance (ANOVA) with repeated-measures. Where a significant main effect was found, Bonferroni post-hoc pair-wise comparisons were determined (Atkinson, 2002). One-way ANOVA with repeated measures were used to compare peak GI symptom scores and bicarbonate kinetic variables (T_{lag}, C_{max}, ΔC_{max}, T_{max} and AUC) between trials. Effect sizes were calculated using partial eta squared (η_p^2) and were described as trivial (<0.20 AU), small (0.20-0.49 AU), moderate (0.50-0.79 AU) or large (≥ 0.80 AU) as previously suggested (Cohen, 1988). The α-level of statistical significance was set at $P < 0.05$ and values for P of "0.000" given by the statistical package were corrected to "< 0.0005" (Kinnear and Gray, 1995). Descriptive data are presented as mean ± SD unless stated otherwise. Data were analysed using the Statistical Package for the Social Sciences (SPSS®) version 25 software.

5.3 Results

5.3.1 Gastrointestinal symptoms

Ingestion form had a significant effect on GI symptoms ($F_{1.4, 17.7} = 10.3$, $P = 0.003$, $\eta_p^2 = 0.44$) with lower GI symptom scores shown during placebo compared to the gelatine ($P = 0.011$), delayed-release ($P = 0.005$) and enteric-coated ($P = 0.02$) trials (Figure 5.1). Enteric-coated capsules also resulted in significantly lower GI symptom scores compared to gelatine (4.8 ± 1.4 AU; 95% CI [1.0-8.6 AU]; $P = 0.025$) but not delayed-release capsules ($P = 0.211$) post-ingestion. Time had no effect on GI symptoms ($F_{1.8, 23.8} = 2.1$, $P = 0.148$, $\eta_p^2 = 0.14$) and there was no significant trial × time interaction ($F_{2.6, 34.0} = 1.8$, $P = 0.180$, $\eta_p^2 = 0.12$).

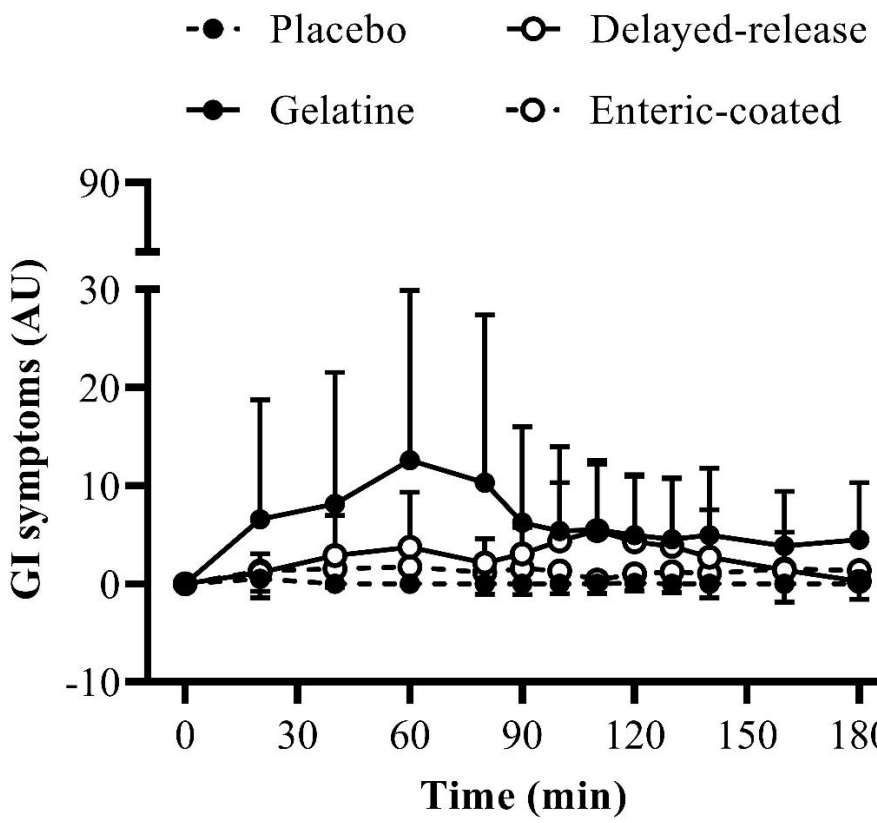

Figure 5.1. Aggregated (mean ± SD) GI symptom scores following the ingestion of placebo and different $NaHCO_3$ ingestion forms. Some error bars are removed for clarity.

The highest individual GI symptom (Table 5.1) occurred between 60 to 90 min post-ingestion (gelatine 94 ± 58 min, delayed-release 91 ± 21 min, enteric-coated 73 ± 34 min) and was significantly earlier with enteric-coated than in the gelatine (P = 0.028) trial. No other differences were shown in the time to reach peak GI symptom scores (P > 0.05) between trials. In total, eleven participants experienced GI symptoms at the point of T_{max} in the gelatine trial compared with seven and one in the delayed-release and enteric-coated trials respectively.

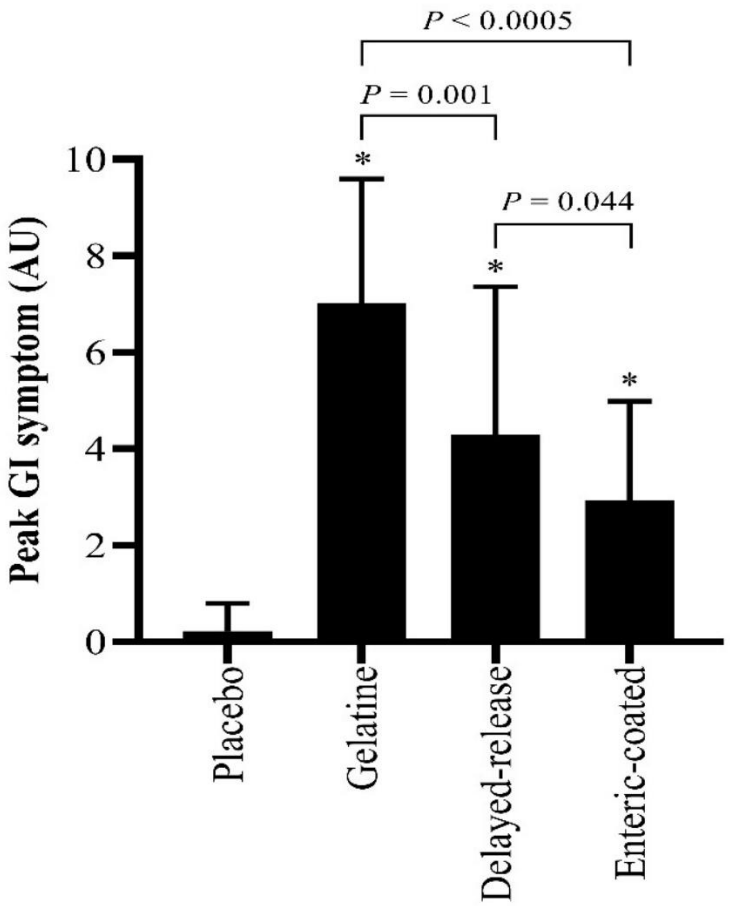

Figure 5.2. Mean (± SD) peak GI symptoms reported following the ingestion of placebo and different $NaHCO_3$ ingestion forms. Peak GI symptom refers to the highest individual symptom reported up to 180 min post-ingestion. *Significantly greater than placebo (P < 0.05)

Table 5.1. Frequency and severity of GI symptoms following $NaHCO_3$ ingestion (n = 14). The most frequent and severe GI symptoms are highlighted in bold.

	Overall		**Placebo**		**Gelatine**		**Delayed-release**		**Enteric-coated**	
Symptoms	%	Mean ± SD	%	Mean ± SD	%	Mean ± SD	%	Mean ± SD	%	Mean ± SD
Stomach bloating	**100.0**	4.4 ± 1.9	0.0	0.0 ± 0.0	**92.9**	3.7 ± 1.8	**78.6**	3.6 ± 2.2	50.0	2.9 ± 1.2
Belching	92.9	3.5 ± 1.4	7.1	**2.0 ± 0.0**	85.7	3.4 ± 1.6	64.3	2.3 ± 1.4	**57.1**	2.3 ± 0.9
Bowel urgency	92.9	5.9 ± 3.0	0.0	0.0 ± 0.0	85.7	5.9 ± 3.1	71.4	3.8 ± 2.3	21.4	3.3 ± 1.5
Stomach ache	85.7	3.5 ± 2.3	**14.3**	1.5 ± 0.7	71.4	3.5 ± 2.6	57.1	2.2 ± 1.5	14.3	1.0 ± 0.0
Stomach cramps	78.6	4.0 ± 2.2	0.0	0.0 ± 0.0	64.3	4.4 ± 2.3	28.6	2.1 ± 0.7	7.1	3.0 ± 0.0
Flatulence	71.4	4.4 ± 2.7	0.0	0.0 ± 0.0	57.1	3.6 ± 2.9	28.6	3.0 ± 1.2	35.7	3.8 ± 2.2
Diarrhoea	64.3	**7.8 ± 3.1**	0.0	0.0 ± 0.0	50.0	**8.2 ± 3.4**	42.9	**6.2 ± 2.7**	7.1	**5.0 ± 0.0**
Nausea	57.1	3.0 ± 2.4	0.0	0.0 ± 0.0	35.7	3.6 ± 2.7	35.7	1.6 ± 1.3	7.1	4.0 ± 0.0
Vomiting	14.3	1.0 ± 1.0	0.0	0.0 ± 0.0	7.1	1.0 ± 0.0	7.1	2.0 ± 0.0	0.0	0.0 ± 0.0
Any	100.0	4.2 ± 0.8	14.3	1.8 ± 0.4	100.0	4.1 ± 1.0	85.7	3.0 ± 1.4	92.9	2.8 ± 1.5

Notes: Overall percentage includes those who reported GI symptoms after at least one $NaHCO_3$ ingestion form.

Table 5.2. Peak GI symptoms reported following different $NaHCO_3$ ingestion forms. Symptom scores are displayed in parentheses.

Participant	Placebo	Gelatine	Delayed-release	Enteric-coated
1	No symptom (0.0)	Bowel urgency (7.0)	Stomach bloating (3.0)	Belching (2.0)
2	No symptom (0.0)	Diarrhoea (10.0)	Diarrhoea (7.0)	Belching (1.0)
3	No symptom (0.0)	Stomach bloating (4.0)	Diarrhoea (5.5)	Diarrhoea (5.0)
4	No symptom (0.0)	Bowel urgency (6.0)	Flatulence (5.0)	Bowel urgency (3.0)
5	No symptom (0.0)	Stomach cramp (6.0)	Bowel urgency (2.0)	Belching (3.0)
6	Stomach ache (2.0)	Diarrhoea (10.0)	Stomach ache (4.5)	Nausea (3.0)
7	No symptom (0.0)	Diarrhoea (10.0)	Diarrhoea (8.0)	Bowel urgency (5.0)
8	No symptom (0.0)	Bowel urgency (4.0)	Stomach cramp (1.2)	Stomach bloating (3.0)
9	No symptom (0.0)	Diarrhoea (10.0)	Stomach bloating (8.0)	Flatulence (6.0)
10	No symptom (0.0)	Nausea (8.0)	Diarrhoea (7.0)	Belching (2.0)
11	Belching (1.0)	Belching (5.0)	Bowel urgency (2.0)	Belching (1.0)
12	No symptom (0.0)	Stomach bloating (4.3)	No symptom (0.0)	No symptom (0.0)
13	No symptom (0.0)	Diarrhoea (10.0)	Diarrhoea (8.5)	Flatulence (5.0)
14	No symptom (0.0)	Stomach cramp (4.0)	Stomach bloating (3.0)	No symptom (0.0)

All fourteen participants experienced at least one GI symptom in the gelatine trial, compared to twelve participants in the delayed-release and enteric-coated trials (Table 5.2). Only two participants reported any GI symptoms in the placebo trial. Stomach bloating (100.0%), belching (92.9%) and bowel urgency (92.9%) were the most common GI symptoms overall, whereas diarrhoea (7.8 ± 3.1 AU) and bowel urgency (5.9 ± 3.0 AU) had the highest severity rating (Table 5.1).

5.3.2 Acid-base responses

Ingestion form had a significant effect on blood [HCO_3^-] ($F_{3.0, 39.0}$ = 53.6, P < 0.0005, η_p^2 = 0.81) and pH ($F_{3.0, 39.0}$ = 50.7, P < 0.0005, η_p^2 = 0.80) (Figure 5.3 and 5.4). Blood [HCO_3^-] and pH were significantly lower with enteric-coated than with gelatine and delayed-release in the post-ingestion phase. In the post-ingestion period, there were significant effects on blood [HCO_3^-] ($F_{3.4, 44.1}$ = 143.8, P < 0.0005, η_p^2 = 0.92) and pH ($F_{5.3, 68.5}$ = 36.3, P < 0.0005, η_p^2 = 0.74). Blood [HCO_3^-] at 110 min was significantly higher than at baseline, 20 and 40 min (P < 0.0005). Blood pH was significantly higher at 140 min than at baseline and 20 min (P < 0.0005). Significant trial × time interactions were shown for blood [HCO_3^-] ($F_{5.2, 67.3}$ = 28.9, P < 0.0005, η_p^2 = 0.69) and pH ($F_{8.3, 108.3}$ = 6.0, P < 0.0005, η_p^2 = 0.32). Figures 5.3 and 5.4 displays significant differences between trials at each time point. No changes in blood [HCO_3^-] or pH were shown for placebo throughout the post-ingestion phase (P > 0.05).

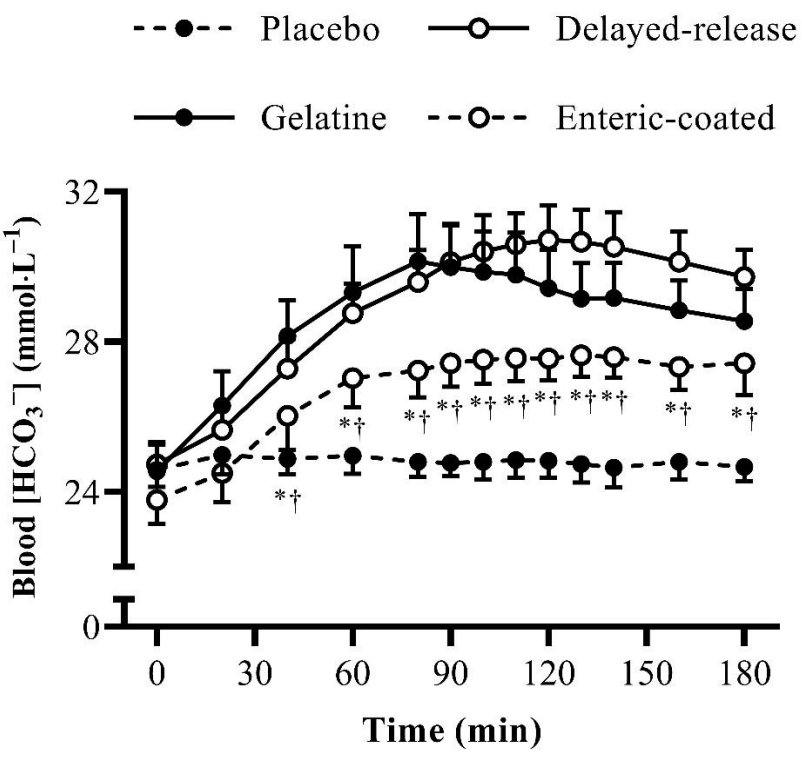

Figure 5.3. Mean (± 95% CI) blood [HCO_3^-] pre- and post-ingestion. *Significant from gelatine trial (P < 0.05). †Significant from delayed-release trial (P < 0.05).

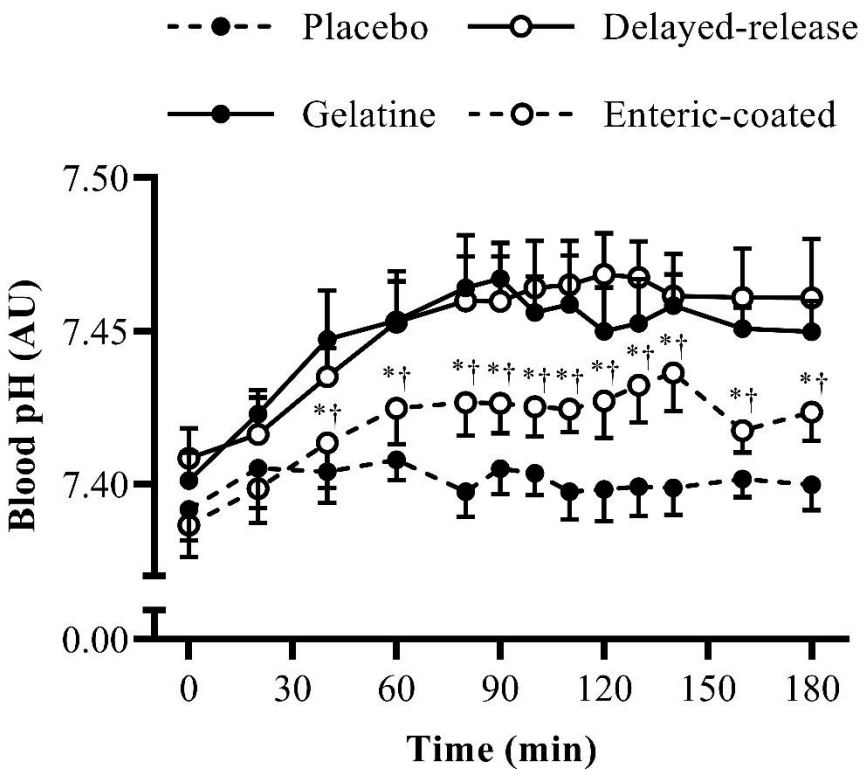

Figure 5.4 Mean (± 95% CI) blood pH pre- and post-ingestion. *Significant from gelatine trial ($P < 0.05$). †Significant from delayed-release trial ($P < 0.05$).

Blood pH peaked at similar time points across trials (GEL 101 ± 30 min, delayed-release 119 ± 23 min, enteric-coated 113 ± 32 min; $P > 0.05$), with no significant differences between trials. Changes in peak blood pH were similar in the gelatine (0.08 ± 0.02 AU) and delayed-release (0.08 ± 0.02 AU) trials, whereas changes with enteric-coated (0.06 ± 0.02) were significantly lower than with gelatine ($P = 0.047$) and delayed-release ($P = 0.047$) $NaHCO_3$.

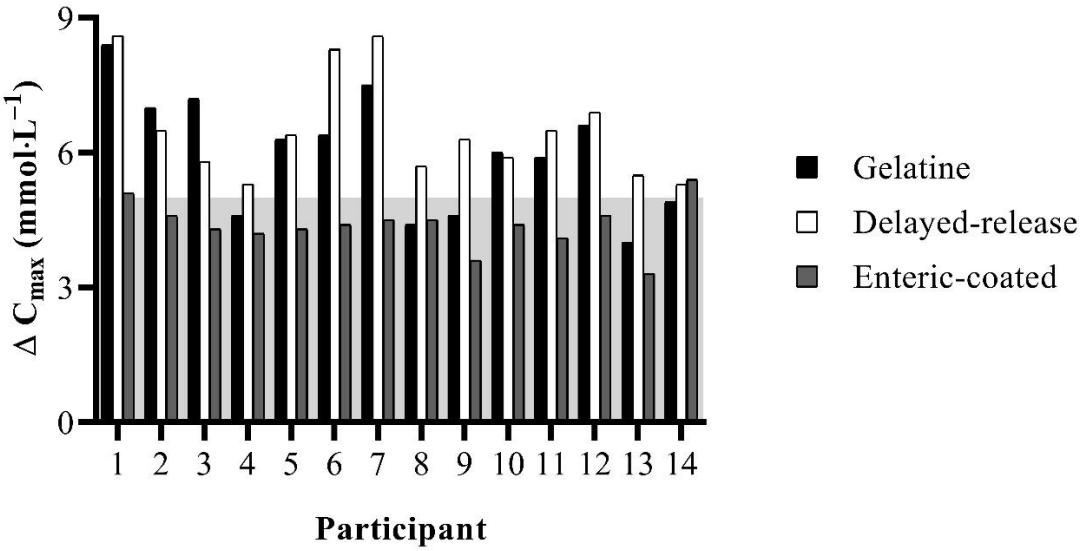

Figure 5.5. Individual changes in blood [HCO_3^-] following ingestion of different $NaHCO_3$ ingestion forms. Grey area indicates 5 mmol·L^{-1} increase in blood [HCO_3^-].

Table 5.3. Within-trial variation in bicarbonate kinetic variables for different $NaHCO_3$ ingestion forms.

	Gelatine		**Delayed-release**		**Enteric-coated**	
Variable	Mean ± SD	CV	Mean ± SD	CV	Mean ± SD	CV
T_{lag} (min)	21 ± 5[b,c]	24.9	30 ± 10[a]	34.6	31 ± 13[a]	41.1
C_{max} (mmol·L^{-1})	30.5 ± 1.9[c]	6.1	31.3 ± 1.4[c]	4.5	28.2 ± 0.9[a,b]	3.3
Δ C_{max} (mmol·L^{-1})	6.0 ± 1.3[c]	22.1	6.5 ± 1.2[c]	17.8	4.4 ± 0.5[a,b]	12.0
T_{max} (min)	95 ± 32[b]	33.5	119 ± 24[a]	20.4	120 ± 35	29.2
AUC (mmol·min·L^{-1})	688 ± 307[c]	44.7	741 ± 237[c]	32.0	343 ± 190[a,b]	55.5

Notes: CV, coefficient of variation; T_{lag}, lag time; C_{max}, peak blood bicarbonate ion concentration; Δ C_{max}, change in peak blood bicarbonate ion concentration; T_{max}, time to reach peak blood bicarbonate ion concentration; AUC, area under the concentration-time curve. [a]Significant from

gelatine trial ($P < 0.05$). [b]Significant from delayed-release trial ($P < 0.05$). [c]Significant from enteric-coated trial ($P < 0.05$). CV was calculated as 100 × (SD/μ).

5.4 Discussion

The present study is the first to investigate the effects of capsule ingestion form on GI disturbances and acid-base balance following $NaHCO_3$ ingestion. The key finding is that enteric-coated $NaHCO_3$ results in fewer and less severe GI symptoms compared to both the gelatine and delayed-release capsules. Furthermore, enteric-coated $NaHCO_3$ resulted in very few participants experiencing GI symptoms at the point of peak blood [HCO_3^-]; a time when exercise is likely to be scheduled for athletes using individualised ingestion strategies (Heibel et al. 2018). Interestingly, blood [HCO_3^-] was increased above 5 mmol·L^{-1} for longer with delayed-release $NaHCO_3$ compared to the gelatine capsules (Figure 5.3), which may provide a greater 'ergogenic window' following supplementation (Carr, Hopkins and Gore, 2011), although this has yet to be confirmed. Altogether, these data suggest that enteric-coated capsules are more effective at attenuating GI symptoms following $NaHCO_3$ ingestion however, delayed-release $NaHCO_3$ maximises extracellular buffering capacity.

In the present study GI symptoms were reported by 85.7% of the participants across all $NaHCO_3$ ingestion trials, which is higher than some studies have previously reported (Sale et al. 2011, Driller et al. 2012, Saunders et al. 2014b). Stomach bloating, belching and bowel urgency were the most common reported GI symptoms overall and although less frequent, the severity of diarrhoea was particularly high (Table 5.1). As expected, GI symptoms decreased following delayed-release and enteric-coated $NaHCO_3$ ingestion compared to the gelatine capsules, therefore suggesting that bypassing the stomach can alleviate many of the symptoms associated with acute bicarbonate loading (Farias de Oliveira, Saunders and Artioli, 2018). In

relation to causes of GI symptoms, these are unlikely to be due to the large number of capsules ingested since only minor symptoms were experienced with the placebo. Instead, GI symptoms can be attributed to the $NaHCO_3$ alone, which also highlights that in some athletes, symptoms are likely to occur with oral delivery despite substantially fewer side-effects with gastro-resistant capsules. Given the relationships observed for GI symptoms between ingestion forms, it appears that some individuals are more prone to GI disturbances than others, although the reasons for this are currently unclear.

Exercise timed with peak alkalosis may optimise performance benefits (Gough et al. 2017a), therefore it is prudent to consider which GI symptoms will occur at this timepoint. While all participants reported at least one GI symptom post-ingestion, only three participants reported GI symptoms at the point when exercise would likely commence. Of these participants, all were experiencing severe diarrhoea (10.0 AU) at the point of peak alkalosis, which may negatively affect subsequent exercise performance (Deb et al. 2018). Nevertheless, it is difficult to suggest which GI symptoms may adversely affect exercise performance based on the current data. Since very few exercise-based studies have quantified GI symptoms, it may be the severity that modulates the effects on performance, rather than GI symptoms per se. This may also explain why some studies have found GI symptoms to have deleterious effects on performance with $NaHCO_3$ supplementation (Cameron et al. 2010, Saunders et al. 2014a, Deb et al. 2018) whereas others have not (Price and Simons, 2010, Miller et al. 2016). To elucidate the effects of GI disturbances on performance, future studies should look to describe the GI symptoms experienced immediately prior to exercise, as well as the athletes perceived readiness to commence.

Blood [HCO_3^-] and pH corresponded to increases typically observed with 300 $mg \cdot kg^{-1}$ body mass $NaHCO_3$ (Matson and Tran, 1993), however this was not observed with enteric-coated $NaHCO_3$. Indeed, enteric-coated capsules reduced the bioavailability of bicarbonate

with changes of ~ 4 mmol·L^{-1} post-ingestion, which may hinder the effect on exercise performance. Bicarbonate anions are actively transported across the intestinal mucosa rather than passively and become saturated (Turnberg et al. 1970), which could explain why blood concentrations are reduced with enteric-coated $NaHCO_3$. Furthermore, as enteric-coated capsules do not disintegrate until reaching the small intestine, the time available for absorption is decreased which may have contributed to reduced blood concentrations. Similar to previous work (Gough et al. 2017b), changes in blood [HCO_3^-] and pH demonstrated a high degree of inter-individual variability following $NaHCO_3$ ingestion (Table 5.3). Interestingly, changes in blood [HCO_3^-] were less variable with enteric-coated capsules which suggests that much of the variation derives from the degree of neutralisation in the stomach. Although blood [HCO_3^-] was similar between gelatine and delayed-released $NaHCO_3$, concentrations were elevated for longer in the delayed-release form. Similarly, more participants reached concentrations that are associated with performance-enhancing effects (Carr, Hopkins and Gore, 2011). Given that higher pre-exercise blood [HCO_3^-] is associated with greater performance enhancements (Matson and Tran, 1993), ingestion form may alter the ergogenic potential of $NaHCO_3$ supplementation.

5.5 Conclusions

In conclusion, the ingestion of $NaHCO_3$ in delayed-release or enteric-coated capsules attenuates GI disturbances following $NaHCO_3$ ingestion. Enteric-coated $NaHCO_3$ is optimal for minimising GI symptoms and may be favourable for those who report GI disturbances following supplementation, even in those who have tried alternative ingestion strategies. Given the dramatic variation in the timing of peak blood [HCO_3^-] (60-180 min) across ingestion forms, it is recommended that athletes continue to adopt an individualised ingestion strategy. Gelatine capsules can be ingested 90 min prior to exercise for athletes without access to a

blood-gas analyser, whereas delayed-release and enteric-coated $NaHCO_3$ can be ingested 90-120 min before exercise. Given that blood buffering capacity was blunted with enteric-coated $NaHCO_3$, future research should look to determine the effects of enteric-coated $NaHCO_3$ on exercise performance.

Chapter 6: The Effects of Enteric-Coated $NaHCO_3$ Supplementation on High-Intensity Exercise Performance in Trained Cyclists

6.1 Introduction

In studies 1 (Chapter 4) and 2 (Chapters 5) it was shown that the prevalence of GI symptoms was high (86-100%), with stomach bloating and diarrhoea being the most frequent and severe symptoms, respectively. Whilst enteric-coated capsules were more affective at reducing GI symptoms compared with delayed-release capsules, the efficacy relating to exercise performance has yet to be questioned. Given that increases in blood [HCO_3^-] were lower with enteric-coated capsules, the subsequent effects on exercise performance warrants further investigation.

High-intensity exercise bouts are impaired by peripheral fatigue, typically as a result of disturbances to intramuscular homeostasis (Thomas et al. 2014). Significant decreases in muscle and blood pH have been observed due to the glycolytic contribution during high-intensity exercise (Hollidge-Horvat et al. 2000). While the mechanisms responsible for the decline in muscular force across the neuromuscular junction are equivocal (Fitts, 2016, Westerblad, 2016), reductions in muscle pH are associated with simultaneous declines in muscle excitability (Cairns and Lindinger, 2008), contractility (Spriet, Matsos and Peters, 1985) and exercise performance (Raymer, 2004). Nutritional strategies that offset these perturbations to acid-base balance have therefore received considerable attention.

Inducing metabolic alkalosis prior to exercise, which can be achieved by oral ingestion of $NaHCO_3$, has been found to improve various performance measures (e.g. power, speed, time-to-completion) during single-bouts of high-intensity exercise (Matson and Tran, 1993, Peart et al. 2011, Lancha Junior et al. 2015). Through increases in extracellular [HCO_3^-], $NaHCO_3$ supplementation can augment buffering capacity (Siegler et al. 2012) and strong ion handling (Raymer, 2004), both of which favour high-intensity exercise performance. Although 0.2 to 0.4 $g \cdot kg^{-1}$ body mass $NaHCO_3$ is generally regarded as ergogenic during high-intensity exercise (McNaughton et al. 2016), GI disturbances can be a problematic side-effect, with some

individuals reporting severe symptoms at the onset of exercise (Burke and Pyne, 2007, Kahle et al. 2013). Whilst some studies have shown that $NaHCO_3$ can improve exercise performance despite GI disturbances (Price and Simons, 2010), there is evidence to suggest that symptoms may compromise the performance-enhancing effects of supplementation (Cameron et al. 2010, Saunders et al. 2014a, Deb et al. 2018). Furthermore, there are claims that athletes may be deterred from supplementing with $NaHCO_3$ due to the risk of adverse side-effects during training and/or competition (Heibel et al. 2018).

Whilst enteric-coated $NaHCO_3$ can reduce GI symptoms post-ingestion as shown in studies 1 and 2, no study to date has investigated the effects on exercise performance. Therefore, it is unknown whether ingesting $NaHCO_3$ in enteric-coated capsules alters the overall ergogenicity of supplementation. Furthermore, knowledge of the performance-enhancing potential of enteric-coated $NaHCO_3$ would help to elucidate the impact of GI symptoms and acid-base balance on exercise performance, as well as improve the practical recommendations for athletes. The aim of the present study therefore, was to determine whether enteric-coated $NaHCO_3$ improves high-intensity exercise performance using an acute loading protocol.

6.2 Methods

6.2.1 Participants

Eleven trained male cyclists (according to DePauw et al. 2013) were recruited for the study (age 32 ± 12 years, body mass 81.5 ± 12.5 kg, height 1.8 ± 0.1 m, $\dot{V}O_{2peak}$ 63.2 ± 4.9 $ml \cdot kg^{-1} \cdot min^{-1}$) based upon sample size estimation. Sample size was determined *a priori* and revealed that eleven participants were required to detect changes (~ 3 s; 1.3%) in time-to-completion between conditions with high statistical power ($\alpha = 0.05$; $\beta = 0.20$). The benchmark for change in performance was chosen as it reflects the difference in time-to-completion between podium

and non-podium position for similar cycling events (Christensen et al. 2017). All participants undertook regular cycling (≥ 3 d·week^{-1}) for at least 5 h·week^{-1} and were free of GI-related disorders. Exclusion criteria included those with hypertension, renal impairment or following a salt-restricted diet, and no participants were ingesting any nutritional supplements or medications at the time of the study. Ethical approval was obtained by the institutional research ethics committee and all participants gave written informed consent to take part in the study.

6.2.2 Experimental design

In a randomised, double-blind, and crossover design, participants attended the laboratory on six occasions, separated by at least 48 h and at the same time of day (0900 h). During the initial visit, participants completed a preliminary test to determine $\dot{V}O_{2peak}$ before familiarisation with the 4 km cycling TT. During the further two visits, individual responses to $NaHCO_3$ ingestion (gelatine and enteric-coated) were established to determine subsequent ingestion timings. Throughout the next three visits, participants performed a maximal 4 km TT (TT) under three different experimental conditions that were administered in a counterbalanced order. Experimental trials involved the consumption of 0.3 g·kg^{-1} body mass of $NaHCO_3$ in either enteric-coated or gelatine capsules, or a placebo containing cornflour prior to the 4 km TT. Participants were instructed to abstain from alcohol and caffeine consumption for 12 h, and strenuous exercise 24 h before each laboratory visit. Water intake was encouraged in the 24 h preceding experimental testing and participants were asked to arrive at the laboratory after an overnight fast and well-hydrated. On arrival to the laboratory, pre-test instructions were confirmed verbally to limit confounding nutritional effects on exercise performance. Physiological (heart rate and blood lactate) and perceptual responses were recorded throughout

the 4 km TT, whereas acid-base balance and GI symptoms were recorded immediately pre- and post-exercise.

6.2.3 Preliminary testing

Participants undertook an incremental exercise test to volitional exhaustion on an electromagnetically-braked cycle ergometer (Lode Excalibur Sport, Groningen, The Netherlands) which confirmed that $\dot{V}O_{2peak}$ was > 55 ml·kg^{-1}·min^{-1}. The protocol involved a 5 min warm-up at 70 W and a self-selected cadence (70–120 rev·min^{-1}), after which the workload increased by 1 W every 2 s (30 W·min^{-1}) until volitional exhaustion. Breath-by-breath gases were measured continuously throughout using a gas analyser (Oxycon Pro™, Jaeger, Germany) whereas heart rate (Polar®, Kempele, Finland) and RPE were recorded each minute (Borg, 1973). The following criterion were used to confirm that $\dot{V}O_{2peak}$ had been reached: (i) heart rate within 10 beats·min^{-1} of age-predicted maximum; (ii) RER > 1.10 arbitrary units (AU); (iii) RPE > 18/20 AU (Midgley et al. 2007). After a period of recovery (30 min), participants performed a 4 km cycling TT to familiarise themselves with the exercise protocol. Individual responses to the ingestion of enteric-coated and gelatine $NaHCO_3$ were established to allow exercise to be scheduled with peak bicarbonate buffering capacity. This method accounts for the inter-individual variability in acid-base kinetics following $NaHCO_3$ ingestion (Jones et al. 2016) and differences between ingestion forms (Hilton, Leach, Hilton, et al. 2019). Semi-nude body mass was recorded (Bod Pod®, Cosmed, Rome, Italy) after bladder evacuation to determine the dose of $NaHCO_3$. Participants then consumed 300 mg·kg^{-1} body mass of $NaHCO_3$ which was administered in either size 0 opaque enteric-coated (Bicarbi™, Nephcentric©, Arizona, USA), or gelatine (Bulk Powders™, Colchester, UK) capsules. Enteric-coated capsules were pre-filled by the manufacturer, whereas gelatine capsules were manually filled by the researcher using a capsule filling device (Capsule Connection LLC,

Arizona, USA), whereas enteric-coated capsules were pre-filled by the manufacturer. Given that each capsule contained 0.65 g of $NaHCO_3$, supplements were administered to the nearest whole capsule. All supplements were checked for accuracy (Ohaus®, Fisher Scientific™, Pennsylvania, USA) prior to administration and were ingested with an equal volume (6 $ml \cdot kg^{-1}$ body mass) of water (Evian®, Danone, Paris, France) within 5 min of commencing ingestion. Fingertip capillary blood samples (95 μL) were drawn pre-ingestion and then every 20 min for 180 min post-ingestion, with 10 min sampling from 80 to 140 min. Fingertip capillary blood samples were collected in heparin-coated glass capillary tubes (Radiometer Medical Ltd, Copenhagen, Denmark) using an aseptic technique and analysed immediately (Radiometer ABL800 BASIC, Copenhagen, Denmark) for blood [HCO_3^-] and pH.

6.2.4 Experimental testing

Upon arrival to the laboratory, participants sat resting for 20 min before a baseline (pre-ingestion) capillary blood sample was taken. Participants then ingested either 300 $mg \cdot kg^{-1}$ body mass of $NaHCO_3$ administered in gelatine or enteric-coated capsules, or a placebo. The opaque gelatine capsules were also used in the placebo trials and an equal number of capsules were given to mask the experimental conditions. Pre-exercise acid-base balance was determined with a further blood sample, after the pre-determined time-to-reach peak blood [HCO_3^-] had passed. All blood samples were analysed immediately for [HCO_3^-] and pH, as well as for blood [Na^+] and [K^+].

6.2.5 Time trials

Participants selected a preferred handlebar and saddle position which was then replicated for all other experimental trials. After a 5 min self-selected warm-up and 3 min rest, participants performed a maximal 4 km cycling TT on an electromagnetically-braked cycle ergometer

(Velotron Pro®, RacerMate™, Seattle, USA) from a static start. Participants were instructed to complete the TT as fast as possible and were free to change gears throughout, although gear ratios were fixed. Visual feedback of cadence, gearing and distance travelled was provided on-screen, although participants were blinded from power output, speed and time elapsed. Strong verbal encouragement was given by the same individual at regular (0.5 km) intervals throughout and no water was provided during the TT. All TTs took place under standardised laboratory conditions (temperature 18-20 °C, humidity 45 ± 5%) and a fan was placed 5-m in front of the cycle ergometer to promote evaporative cooling. Participants undertook a 5 min cool-down at a self-selected workload immediately after completion of the TT.

6.2.6 Physiological and perceptual measures

During each TT, blood La^- was measured pre- and post-exercise, and every 1-km throughout using a portable lactate monitor (Lactate Pro 2, Arkray, Japan). At the same time points, lower-limb ratings of perceived exertion (RPE-L) and RPE were recorded using a 6–20 scale (Borg, 1973), whereas perceived ratings of fatigue (ROF) were recorded on a 10-point Likert scale (Micklewrigtht et al. 2017). Heart rate was measured pre- and post-exercise, and every 0.5-km throughout the TT (Polar®, Kempele, Finland). Symptoms of GI distress were recorded immediately pre-exercise using an adapted GI symptom questionnaire (Carr et al. 2011) as previously described. Symptom terminology was explained to participants before the experimental trials commenced to ensure consistency in the reporting of symptoms.

6.2.7 Statistical analyses

Data normality was assessed using the Shapiro–Wilk test and by visual inspection of the normality plots (Grafen and Hails, 2002). One-way analysis of variance (ANOVA) for repeated-measures were used to compare time-to-completion and GI symptom scores. All

cardiovascular (heart rate), blood ([HCO_3^-], pH, [La^-], [Na^+], [K^+] and [Cl^-]), perceptual (RPE, RPE-L and ROF) and performance (power) variables were analysed using two-way (condition × time) ANOVA for repeated-measures. Where a significant main effect was found, Bonferroni-adjusted post-hoc paired comparisons were determined (Atkinson, 2002). Effect sizes were reported as partial eta squared (η_p^2) for one- and two-way ANOVA, whereas Hedge's g and 95% CI were calculated for paired comparisons. Effects were discussed in relation to the relevant literature (Thompson, 2007) and described as trivial (<0.20), small (0.20-0.49), moderate (0.50-0.79) or large (≥ 0.80) as previously suggested (Cohen, 1988). Statistical significance was set at $P < 0.05$ and values for P of "0.000" given by the statistical package were corrected to "< 0.0005" (Kinnear and Gray, 1995). Descriptive data are presented as mean ± SD throughout. Data were analysed using the Statistical Package for the Social Sciences version 25 software (IBM®, Chicago, USA), whereas sample size was calculated using GPower® version 3.1.9.2 (Faul et al. 2007).

6.3 Results

6.3.1 Exercise performance

There was a significant improvement in time-to-completion (Figure 6.1) in the $NaHCO_3$ trials compared with the placebo ($F_{2.0, 20.0} = 10.6$, $P = 0.001$, $\eta_p^2 = 0.52$). Time-to-completion was significantly faster with enteric-coated (mean difference = 8.6 s [−2.3%], $P = 0.044$, 95% CI [0.2, 16.9 s], $g = 0.41$) and gelatine (mean difference = 9.6 s [−2.6%], $P = 0.004$, 95% CI [3.4, 15.9 s], $g = 0.50$) $NaHCO_3$ compared with the placebo, but there was no difference between enteric-coated and gelatine $NaHCO_3$ (mean difference = −1.1 s, $P = 1.00$, 95% CI [–5.7, 3.5 s], $g = -0.1$). Acute bicarbonate loading had a significant effect on power output ($F_{2.0, 20.0} = 8.8$, $P = 0.002$, $\eta_p^2 = 0.47$; Figure 6.1), with higher values in the gelatine trial when compared with the placebo ($P = 0.016$). No further differences in power output were shown between trials (P

> 0.05). There was significant variation in power output across the TT ($F_{1.4, 14.1}$ = 12.8, P = 0.002, η_p^2 = 0.56) with power output declining between 1 and 2 km (P = 0.001) before reaching a plateau (P = 0.123) at 3 km, followed by an increase towards 4 km (P = 0.026). Pacing strategies were similar between conditions (Figure 6.1), with no significant condition × time interaction ($F_{2.6, 26.1}$ = 0.4, P = 0.746, η_p^2 = 0.04). No order effect on TT performance was shown given that neither time-to-completion nor power output differed between the first to last trial (all P > 0.05).

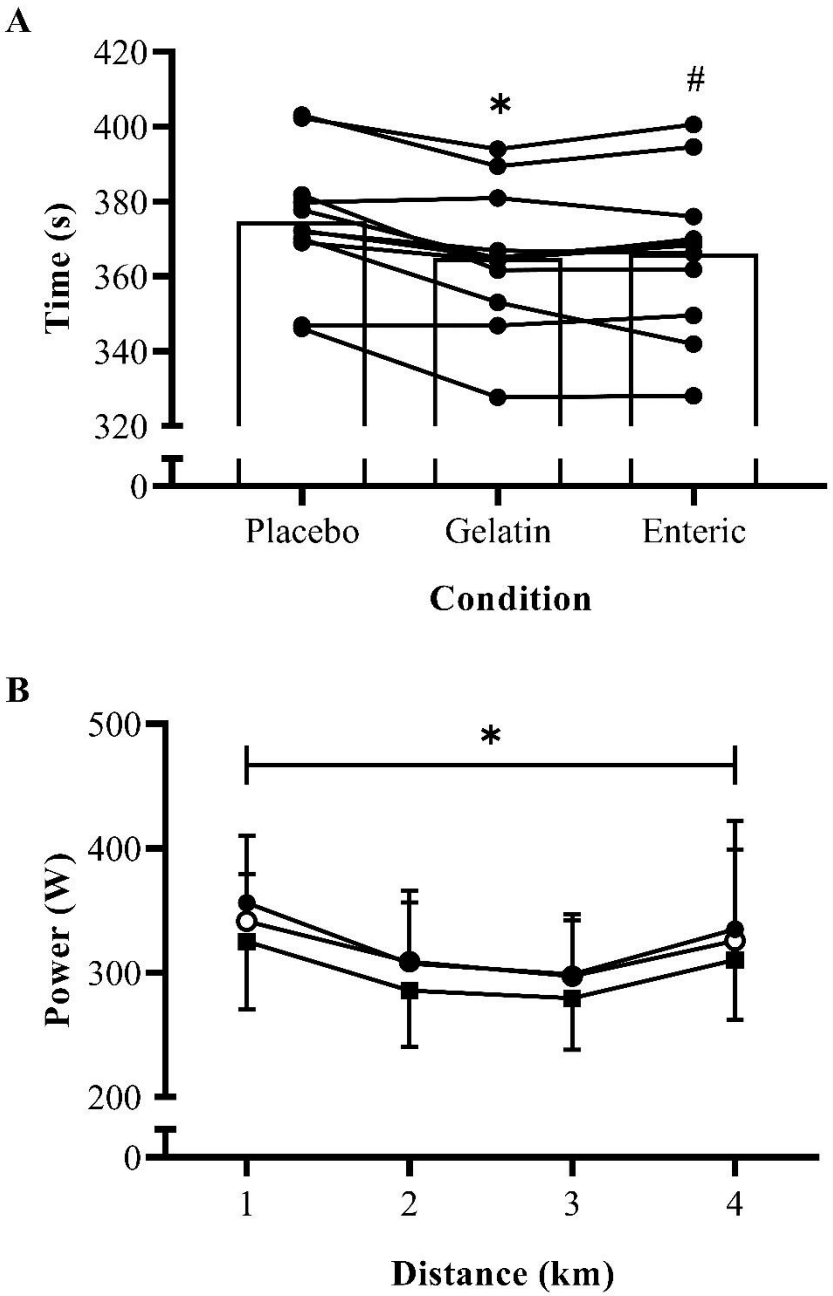

Figure 6.1. Mean (± SD) time-to-completion (A) and power output (B) following the ingestion of 300 mg·kg^{-1} body mass $NaHCO_3$ in gelatine (●) or enteric-coated (○) capsules, or a placebo (■). Individual responses are given for time-to-completion. *Significant difference between gelatine $NaHCO_3$ and placebo ($P < 0.05$). #Significant difference between enteric-coated $NaHCO_3$ and placebo ($P < 0.05$).

6.3.2 Acid–base balance

Blood [HCO_3^-] was significantly higher in the $NaHCO_3$ conditions compared with the placebo ($F_{2.0, 20.0} = 23.5$, $P < 0.0005$, $\eta_p^2 = 0.70$, Figure 6.2), with no difference between enteric-coated and gelatine capsules ($P = 1.0$). Blood [HCO_3^-] increased pre-exercise ($P < 0.0005$) followed by a decrease post-exercise ($P < 0.0005$), with a condition × time interaction ($F_{4.0, 40.0} = 48.2$, $P < 0.0005$, $\eta_p^2 = 0.83$). Pre-exercise blood [HCO_3^-] was significantly higher in the enteric-coated (3.8 ± 1.0 mmol·L^{-1}, $P < 0.0005$, 95% CI [3.0, 4.7 mmol·L^{-1}]) and gelatine (5.6 ± 1.5 mmol·L^{-1}, $P < 0.0005$, 95% CI [4.3, 6.3 mmol·L^{-1}]) conditions compared with the placebo. Furthermore, blood [HCO_3^-] was significantly lower with enteric-coated compared with gelatine capsules pre-exercise (mean difference = 1.8 mmol·L^{-1}, $P = 0.012$, 95% CI [0.4, 3.3 mmol·L^{-1}).

Blood pH was significantly higher in the $NaHCO_3$ conditions compared with the placebo ($F_{2.0, 20.0} = 14.6$, $P < 0.0005$, $\eta_p^2 = 0.59$, Figure 6.2), with no difference between enteric-coated and gelatine capsules ($P = 1.0$). Blood pH increased pre-exercise ($P < 0.0005$) followed by a decrease post-exercise ($P < 0.0005$), with a condition × time interaction ($F_{2.0, 19.7} = 48.2$, $P = 0.001$, $\eta_p^2 = 0.55$). Pre-exercise blood pH was significantly higher in the enteric-coated (0.038 ± 0.016 AU, $P < 0.0005$, 95% CI [0.024, 0.052 AU]) and gelatine (0.074 ± 0.019 AU, $P < 0.0005$, 95% CI [0.058, 0.091 AU]) conditions compared with the placebo. Blood pH was also significantly lower with enteric-coated compared with gelatine capsules pre-exercise (mean difference = 0.037 AU, $P = 0.001$, 95% CI [0.018, 0.055 AU]).

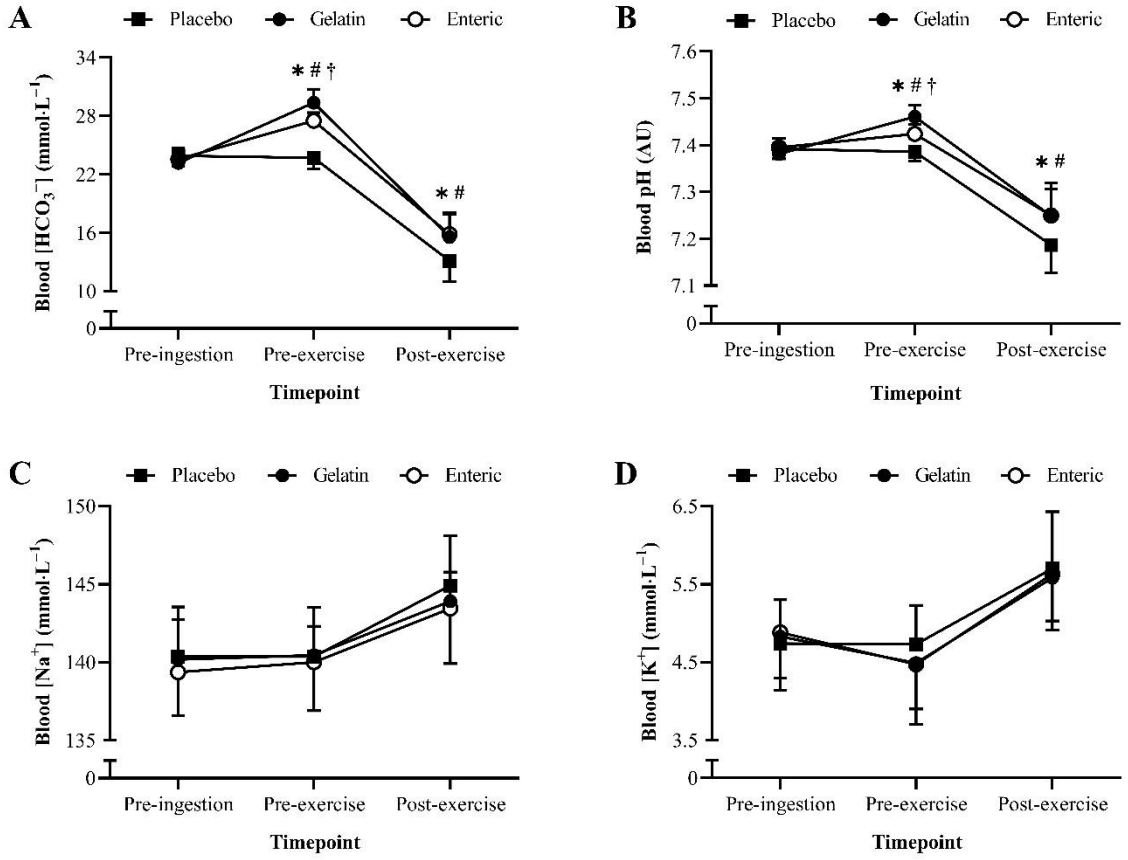

Figure 6.2. Mean (± SD) blood [HCO_3^-] (A), pH (B), [Na^+] (C) and [K^+] (D) pre-ingestion, pre-exercise (post-ingestion) and post-exercise. *****Significant difference between gelatine $NaHCO_3$ and placebo ($P < 0.05$). **#**Significant difference between enteric-coated $NaHCO_3$ and placebo ($P < 0.05$). **†**Significant difference between gelatine and enteric-coated $NaHCO_3$ ($P < 0.05$).

6.3.3 Electrolyte responses

Acute bicarbonate loading did not alter blood [Na^+] ($F_{2.0, 20.0} = 1.0$, $P = 0.394$, $\eta_p^2 = 0.09$, Figure 6.2), although there were significant increases shown post-exercise ($F_{2.0, 20.0} = 20.5$, $P < 0.0005$, $\eta_p^2 = 0.67$). No condition × time interaction was found for blood [Na^+] ($F_{4.0, 40.0} = 0.3$, $P = 0.850$, $\eta_p^2 = 0.03$). Similarly, $NaHCO_3$ ingestion did not alter blood [K^+] ($F_{2.0, 20.0} = 0.2$, $P =$

0.848, η_p^2 = 0.02, Fig. 2d) despite significant decreases post-exercise ($F_{2.0,\ 20.0}$ = 41.1, P < 0.0005, η_p^2 = 0.80), with no condition × time interaction ($F_{4.0,\ 40.0}$ = 0.6, P = 0.660, η_p^2 = 0.06).

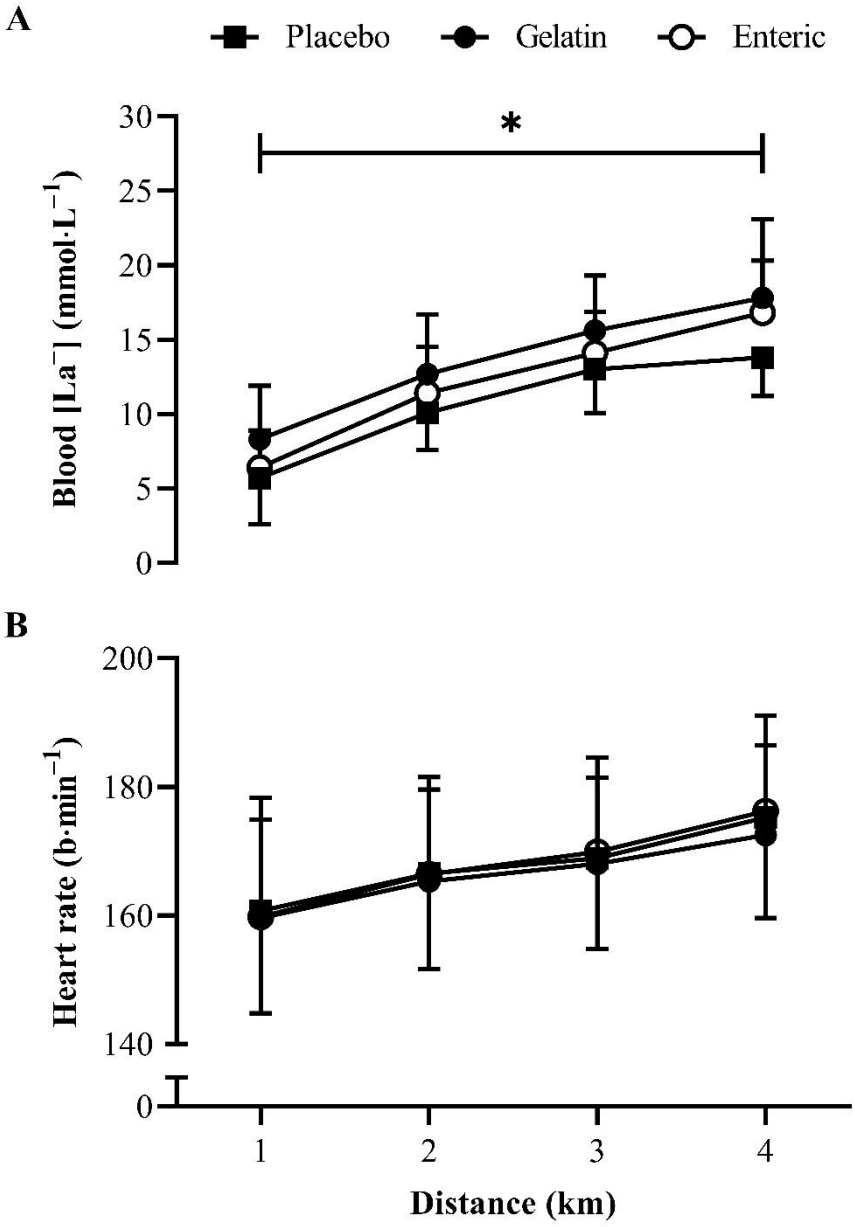

Figure 6.3 Mean (± SD) blood [La–] (A) and heart rate (B) response during the 4 km TT following the ingestion of 300 mg·kg^{-1} body mass $NaHCO_3$ in gelatine (●) or enteric-coated (○) capsules, or a placebo (■). *Significant difference between gelatine $NaHCO_3$ and placebo (P<0.05).

6.3.4 Physiological and perceptual responses

Blood [La^-] ($F_{2.0, 20}$ = 7.7, P = 0.003, η_p^2 = 0.43; Fig. 3a) were significantly greater in the gelatine trial compared with the placebo (mean difference = 2.4 mmol·L^{-1}, P = 0.003, 95% CI [0.9, 3.8 s], g = 0.90). No further differences in lactate responses were shown between conditions (P > 0.05), although blood [La^-] progressively increased during all TT's ($F_{1.4, 13.9}$ = 127.3, P < 0.0005, η_p^2 = 0.93), without a condition × time interaction ($F_{2.5, 25.2}$ = 2.0, P = 0.152, η_p^2 = 0.16). Heart rate progressively increased throughout the 4 km TT ($F_{1.1, 10.9}$ = 43.8, P < 0.0005, η_p^2 = 0.81; Figure 6.3), although no significant differences were shown between conditions ($F_{2.0, 20}$ = 0.7, P = 0.491, η_p^2 = 0.07), nor was there a significant condition × time interaction (P = 0.385). Despite improvements in TT performance in both $NaHCO_3$ conditions, there were no differences in neither RPE ($F_{2.0, 20.0}$ = 2.2, P = 0.137, η_p^2 = 0.18), RPE-L ($F_{2.0, 20.0}$ = 0.2, P = 0.841, η_p^2 = 0.02) nor ROF ($F_{2.0, 20.0}$ = 3.5, P = 0.05, η_p^2 = 0.26) between conditions, although there were significant increases in RPE ($F_{3.0, 30.0}$ = 63.2, P < 0.0005, η_p^2 = 0.86), RPE-L ($F_{1.4, 14.4}$ = 45.2, P < 0.0005, η_p^2 = 0.82) and ROF ($F_{1.2, 12.4}$ = 2.2, P < 0.0005, η_p^2 = 0.87) during the TT (Table 6.1). No significant condition × time interactions were shown for neither RPE ($F_{6.0, 60.0}$ = 0.9, P = 0.524, η_p^2 = 0.08), RPE-L ($F_{6.0, 60.0}$ = 0.4, P = 0.893, η_p^2 = 0.04) nor ROF ($F_{6.0, 60.0}$ = 0.8, P = 0.583, η_p^2 = 0.07).

Table 6.1. Mean (± SD) perceptual responses during the 4 km TT.

	Condition		
	Placebo	Gelatin	Enteric
RPE (AU)			
1-km	11.6 ± 1.9	11.5 ± 2.8	12.5 ± 2.0
2-km	13.3 ± 2.2	12.4 ± 2.5	14.0 ± 1.7*
3-km	14.8 ± 2.2*	13.8 ± 1.8	15.2 ± 2.1*
4 km	16.6 ± 2.3*	16.0 ± 2.8*	16.6 ± 2.2*
RPE-L (AU)			
1-km	13.5 ± 2.7	13.6 ± 2.2	13.7 ± 2.1
2-km	14.7 ± 2.5	14.6 ± 2.2*	15.0 ± 1.7
3-km	16.1 ± 1.9*	15.9 ± 2.0*	16.2 ± 1.6*
4 km	17.3 ± 2.4	17.8 ± 1.7*	18.0 ± 2.1*
ROF (AU)			
1-km	3.9 ± 1.8	3.2 ± 1.3	3.5 ± 1.1
2-km	5.0 ± 1.4*	4.7 ± 0.9*	5.2 ± 1.0*
3-km	5.8 ± 1.1*	5.4 ± 1.2	6.2 ± 1.3
4 km	7.5 ± 1.3*	6.6 ± 1.2*	7.5 ± 1.4*

*Denotes a significant difference from the previous timepoint ($P < 0.05$).

6.3.5 Gastrointestinal symptoms

No GI symptoms were reported pre-ingestion in all conditions. No participants reported GI symptoms pre-exercise with the placebo, whereas fewer participants experienced symptoms with enteric-coated (n = 7) compared to gelatine (n = 3) $NaHCO_3$. Pre-exercise GI symptom scores were significantly higher following gelatine $NaHCO_3$ (3.6 ± 3.9 AU) compared with placebo (P = 0.043), with no difference between enteric-coated $NaHCO_3$ (1.0 ± 1.7 AU) and placebo (P = 0.324). Furthermore, pre-exercise GI symptoms were less severe with enteric-coated $NaHCO_3$ compared to gelatine at the individual level (Table 6.2), although group symptom scores were not significantly different (P = 0.211) between enteric-coated and gelatine capsules (mean difference = 2.6 AU, P = 0.211, 95% CI [1.1, 6.2 AU]).

Table 6.2. Individual GI symptom scores immediately before exercise. Symptoms are displayed in bold for clarity and scores are displayed in parentheses.

	Condition		
	Placebo	Gelatin	Enteric
Participant			
1	No symptom (0.0)	No symptom (0.0)	No symptom (0.0)
2	No symptom (0.0)	**Diarrhoea (10.0)**	No symptom (0.0)
3	No symptom (0.0)	**Stomach ache (1.3)**	No symptom (0.0)
4	No symptom (0.0)	**Stomach cramp (1.5)**	No symptom (0.0)
5	No symptom (0.0)	No symptom (0.0)	**Flatulence (5.0)**
6	No symptom (0.0)	**Diarrhoea (6.0)**	No symptom (0.0)
7	No symptom (0.0)	**Bloating (5.0)**	**Bloating (3.0)**
8	No symptom (0.0)	No symptom (0.0)	No symptom (0.0)
9	No symptom (0.0)	**Bowel urgency (5.0)**	No symptom (0.0)
10	No symptom (0.0)	**Diarrhoea (10.0)**	**Bloating (2.0)**
11	No symptom (0.0)	No symptom (0.0)	No symptom (0.0)

6.4. Discussion

This is the first study to investigate the effect of enteric-coated $NaHCO_3$ supplementation on exercise performance, specifically that which would typically benefit from extracellular

buffering agents. The main finding of this study was that ingesting enteric-coated $NaHCO_3$ prior to exercise improved (~ 2.3%) subsequent 4 km cycling TT performance among trained cyclists. Despite inducing a lower degree of metabolic alkalosis with enteric-coated $NaHCO_3$ (Figure 6.2), there were no differences in exercise performance compared with a standard ingestion form (i.e. gelatine capsules). Furthermore, enteric-coated $NaHCO_3$ reduced GI symptoms experienced immediately before exercise compared with gelatine capsules (Table 6.2), although subjective ratings of GI symptoms in this sample were low. When taken together, these data suggest that enteric-coated $NaHCO_3$ improves high-intensity cycling performance in those with mild to moderate GI symptoms. However, the effects of enteric-coated $NaHCO_3$ on exercise performance could be greater in those who experience more severe GI symptoms at the onset of exercise, although this warrants further investigation. Athletes who experience GI side-effects following acute bicarbonate loading may therefore benefit from enteric-coated $NaHCO_3$ supplementation prior to exercise.

Numerous studies have investigated the effects of $NaHCO_3$ on simulated high-intensity TT events with equivocal outcomes (Callahan et al. 2017, Gough et al. 2018). Where some studies have reported performance improvements (Gough et al. 2018) others have reported no benefit (Callahan et al. 2017, Correia-Oliveira et al. 2017) following supplementation. This disparity between studies could be explained by the timing of supplementation, given that the current study demonstrated positive outcomes when exercise was timed with peak alkalosis. Studies that have reported no effect of $NaHCO_3$ ingestion during similar exercise protocols have administered the supplement at a standardised time (Callahan et al. 2017; Correia-Oliveira et al. 2017) despite considerable variability in the time taken to reach metabolic alkalosis (Jones et al. 2016). Time between ingestion and the onset of exercise largely determines the degree of metabolic alkalosis in terms of blood [HCO_3^-] and pH (Heibel et al. 2018), which in turn, may influence the ergogenicity of $NaHCO_3$ supplementation (Carr et al. 2011). Interestingly, the

effect of $NaHCO_3$ on exercise performance in the present study was mediated by the ingestion form, with a *small* to *moderate* effect on time-to-completion (2.3–2.6%) with gelatine and enteric-coated $NaHCO_3$, respectively. The present study reported a mean 5.6 $mmol \cdot L^{-1}$ increase in blood [HCO_3^-] with gelatine compared to placebo, which is lower than the 3.8 $mmol \cdot L^{-1}$ increase observed with the enteric-coated capsules. This finding is consistent with previous studies that have investigated the acid-base kinetics following $NaHCO_3$ ingestion (Hilton, Leach, Hilton, et al. 2019) , which could account for the difference in effect size observed in the present study. Nevertheless, exercise performance still improved with enteric-coated $NaHCO_3$ supplementation, which questions the 5-6 $mmol \cdot L^{-1}$ threshold suggested to improve performance (Heibel et al. 2018). Indeed, the improvements in 4 km cycling TT performance in the present study are similar to previous studies, despite a greater blood [HCO_3^-] reported by others (Gough et al. 2018). While individualising the timing of supplementation may increase the likelihood of commencing exercise with greater blood buffering capacity, it is not clear that utilising an individualised strategy maximises the ergogenicity of $NaHCO_3$ supplementation. Given this disparity between studies, it is unlikely that timing is the only factor modulating the ergogenicity of $NaHCO_3$ during high-intensity exercise. However, further research should look to compare the effects of an individualised and standardised ingestion time on subsequent performance, particularly when standardised times are based on existing group data.

Given that enteric-coated $NaHCO_3$ improves exercise performance among those with mild to moderate GI symptoms, the effects on exercise performance may be enhanced among those with more severe GI symptoms at the onset of exercise. While GI side-effects were significantly reduced in some individuals in the current study (Table 6.2), numerous individuals did not report symptoms at the onset of exercise. Although ergogenic doses (~ 0.3 $g \cdot kg^{-1}$ body mass) of $NaHCO_3$ may induce GI side-effects, symptoms may not necessarily be timed with

exercise performance. This is consistent with previous studies (Hilton, Leach, Hilton, et al. 2019, Hilton, Leach, Sparks, et al. 2019) demonstrating the reduced incidence of GI symptoms at the time of peak alkalosis, despite severe symptoms at other timepoints. It is therefore difficult to elucidate whether GI symptoms can negate the ergogenic effects of $NaHCO_3$ supplementation from the current data, since the overall incidence and severity of GI symptoms was low. Nevertheless, GI side-effects may hinder high-intensity exercise performance or dampen the ergogenic effects of $NaHCO_3$ supplementation (Saunders et al. 2014a). Further research should therefore examine the effects of enteric-coated $NaHCO_3$ supplementation in those who typically report moderate to severe GI disturbances at the onset of exercise, as the effects may be greater among these individuals.

While psychological indicators of perceived exertion and fatigue increased during exercise, no differences were reported between the placebo and $NaHCO_3$ conditions (Table 6.1), suggesting an alternative mechanism other than reductions in afferent feedback to the central nervous system (Siegler and Marshall, 2015). Nevertheless, this finding indicates the enhancements in power output were attained at a relatively similar RPE when supplementing with $NaHCO_3$. Similarly, despite distinct changes in blood $[Na^+]$ and $[K^+]$ during exercise, no differences were observed between $NaHCO_3$ and placebo (Figure 6.2). Changes in these strong ions can impair muscle excitability (Cairns and Lindinger, 2008), therefore suggesting that improvements in performance were not due to ionic shifts in $[Na^+]$ and $[K^+]$ associated with enhanced contractility. Nevertheless, enhanced muscle contractile function cannot be dismissed as a potential mechanism, as altered calcium handling can improve mechanical efficiency (Siegler and Marshall, 2015), although this cannot be elucidated from the current study. Alternatively, given that pre-exercise blood $[HCO_3^-]$ and pH were greater in the $NaHCO_3$ conditions compared to placebo, the performance improvements observed in the current study may be attributed to increases in extracellular buffering capacity. Reinforced

extracellular HCO_3^- are suggested to promote H^+ efflux from intramuscular to extracellular regions through increases in monocarboxylate transporter activity, which maintains muscle pH during exercise (Bishop et al. 2008). Given the delayed onset of intramuscular acidosis, $NaHCO_3$ promotes glycolytic enzyme activity and flux, as indicated through increases in muscle glycogen utilisation and lactate concentrations (Hollidge-Horvat et al. 2000). Although muscle pH and lactate were not measured in the current study, increases in muscle pH and lactate efflux have been observed during exercise following $NaHCO_3$ supplementation (Costill et al. 1984). Augmenting glycolytic flux may have therefore permitted exercise at higher intensities and could explain the performance improvements observed in the current study. This would account for the greater blood [La^-] observed with gelatine $NaHCO_3$, although the increases observed with enteric-coated capsules did not reach significance (Figure 6.3). Given that monocarboxylate transporters 1- and 4 are stimulated by the intra- to extracellular [H^+] gradient, the greater extracellular pH observed with gelatine capsules may have upregulated the co-transport of H^+ and La^- to a greater extent and could account for differences in the ergogenic effect size (0.3%). This may also explain why power output was greater when $NaHCO_3$ was given in gelatine capsules (Figure 6.1), although this did not result in greater overall performance times compared to enteric-coated capsules. Therefore, the current evidence suggests that while pre-exercise blood [HCO_3^-] does not determine the overall ergogenicity of $NaHCO_3$ supplementation, the magnitude of such effects may be increased by a greater degree of metabolic alkalosis.

6.5 Conclusions

In summary, this study is the first to demonstrate that 0.3 $g \cdot kg^{-1}$ body mass of enteric-coated $NaHCO_3$ improves high-intensity exercise performance when timed with peak alkalosis. This study also provides novel data highlighting that ingestion form (e.g gelatine or enteric-coated

capsules) can mediate the effects on exercise performance, potentially through the degree of induced alkalosis. In order to understand the implications of GI side-effects on exercise performance, further research should compare the effects of enteric-coated $NaHCO_3$ supplementation on exercise performance in those who experience severe symptoms immediately before exercise, particularly as GI disturbances may be ergolytic among these individuals. Furthermore, given the growing range of ingestion forms commercially available to athletes (e.g. solution, gelatine capsules, gastro-resistant capsules), future studies should compare the effects on exercise performance. Nonetheless, acute enteric-coated $NaHCO_3$ consumption improves 4 km TT performance and therefore, may offer an appropriate ergogenic strategy for those who experience GI side-effects following supplementation.

Chapter 7: Synthesis of findings

The aim of this thesis was to investigate the effects of gastro-resistant $NaHCO_3$ supplementation on markers of acid-base balance, GI symptoms and exercise performance. A summary of the outcomes reported in each experimental chapter are provided below:

Study 1 (Chapter 4)

The aim of this study was to determine whether 300 $mg \cdot kg^{-1}$ body mass of delayed-release $NaHCO_3$ could mitigate GI distress and alter acid-base balance compared with a solution. This study was the first randomised control trial investigating the effects of high-dose (300 $mg \cdot kg^{-1}$ body mass) gastro-resistant $NaHCO_3$ in humans. Delayed-release $NaHCO_3$ appeared to postpone the release of $NaHCO_3$ and subsequently increase the time-to-reach metabolic alkalosis. Furthermore, delayed-release $NaHCO_3$ mitigated the prevalence and severity of GI side-effects, although symptoms were still reported by some individuals. These findings are therefore the first to demonstrate that bypassing the stomach, at least in part, can reduce GI side-effects associated with acute bicarbonate loading. In addition, this study revealed that altering the ingestion form can affect $NaHCO_3$ release and subsequent bicarbonate kinetics and acid-base balance.

Study 2 (Chapter 5)

The aim of this study was to investigate whether capsule composition affects GI side-effects and acid-base balance following acute $NaHCO_3$ supplementation, potentially through altering the site of absorption across the GI tract. Symptoms of GI distress were lowest following enteric-coated $NaHCO_3$ consumption and decreased as the gastro-resistant properties of the capsules increased. This demonstrates that enteric-coated $NaHCO_3$ minimises GI symptoms associated with $NaHCO_3$ consumption, which may benefit athletes who report GI side-effects

due to supplementation. Nevertheless, [HCO_3^-] availability was lowest following the consumption of enteric-coated $NaHCO_3$ which could alter the ergogenicity of supplementation.

Study 3 (Chapter 6)

The aim of this study was to investigate whether enteric-coated $NaHCO_3$ supplementation could improve high-intensity exercise performance during a 4 km cycling TT in trained male cyclists. This study was the first randomised control trial investigating the efficacy of enteric-coated $NaHCO_3$ consumption on exercise performance, which used an acute 300 $mg \cdot kg^{-1}$ body mass dosing protocol. Enteric-coated $NaHCO_3$ supplementation provided a small ergogenic benefit, with a mean 9 s (2.3 ± 2.6%) time improvement when compared to the placebo. Furthermore, performance improvements were similar when $NaHCO_3$ was administered in gelatine capsules, with a mean 10 s (2.6 ± 2.0%) time improvement when compared to the placebo. Nevertheless, ingestion form altered the magnitude of effect between performances, which could be mediated through pre-exercise blood [HCO_3^-] and pH.

The following sections are written to further discuss the general findings of this thesis, with limitations, practical implications and future directions provided[.

7.1 Gastrointestinal symptoms

In the experimental chapters (4, 5 and 6) $NaHCO_3$ was generally not well tolerated given that most participants reported GI side-effects. In the context of sport however, athletes may be prepared to experience GI symptoms in the hope that $NaHCO_3$ supplementation will improve exercise performance. This is likely to depend on individual factors, such as the severity of symptoms and the performance level of the athlete (e.g. novice to elite). As a result, practitioners should be aware that $NaHCO_3$ supplementation may cause GI symptoms that athletes may not deem tolerable and warrants an individualised approach. It is therefore

recommended that athletes continue to monitor GI responses to this supplement outside of competition, such as during pre-season or training.

7.1.1 Prevalence of gastrointestinal symptoms with sodium bicarbonate supplementation

The oral administration of $NaHCO_3$ has long been associated with acute GI side-effects (Burke and Pyne, 2007). Whilst GI symptoms vary between individuals, nausea, flatulence, stomach cramp, belching, stomach ache, bowel urgency, diarrhoea, vomiting and stomach bloating are among those typically reported by athletes (Miller et al. 2016). These symptoms are generally considered to be those of the upper GI tract (e.g. belching, nausea and vomiting) and the lower GI tract (e.g. flatulence, bowel urgency and diarrhoea), although the exact prevalence remains unclear. In Chapters 4 and 5, a high proportion (86-100%) of recreationally trained individuals reported GI symptoms following $NaHCO_3$ supplementation irrespective of the ingestion form used, which is similar to that reported in a small group of well-trained athletes (Carr, Gore and Dawson, 2011). This highlights the potential widespread impact of $NaHCO_3$ supplementation on GI side-effects when administering a 300 $mg \cdot kg^{-1}$ body mass dose, which could explain why the use of $NaHCO_3$ is low compared with other nutritional supplements (Knapik et al. 2016). Nonetheless, this figure is greater to that reported in some studies using an acute loading protocol, with reported prevalence rates between 22-38% (McNaughton, Siegler and Midgley, 2008, Driller et al. 2012). Variations between studies could be due to numerous factors, such as co-ingestion with a small carbohydrate-based meal (1.5 $g \cdot kg^{-1}$ body mass), which have been shown to mitigate GI symptoms, and ingestion with a larger volume of water (14 $ml \cdot kg^{-1}$ body mass) which can exacerbate symptoms (Carr et al. 2011). Methodological factors may also contribute to the variation in GI symptoms reported between studies, such as differences in the questionnaire used to measure symptoms and the criteria used to identify a symptom.

Furthermore, some studies have measured the time course of GI symptoms whereas other have recorded symptoms at a solitary timepoint, which makes comparison between these studies difficult. Longer sampling periods may also increase the incidence of GI symptoms given that side-effects can present up to 24 h post-ingestion (Cameron et al. 2010). Symptoms may also be greater when $NaHCO_3$ is ingested over shorter (≤ 30 min) compared with longer (30-60 min) time periods, although this warrants further investigation.

7.2 Implications of gastro-resistant sodium bicarbonate

7.2.1 Gastrointestinal symptoms

Numerous ingestion strategies have been employed to mitigate GI side-effects associated with acute bicarbonate loading, including chronic (McNaughton and Thompson, 2001), multi-day (Mueller et al. 2013) and split-dose (Sale et al. 2011, Saunders et al. 2014b). Given that no study has investigated the effects of these strategies on GI side-effects in isolation, it is difficult to draw firm conclusions on the efficacy relating to symptomology. Nevertheless, it appears that adopting these protocols can still lead to severe GI symptoms in some individuals, which may even limit the performance-enhancing effects of supplementation (Saunders et al. 2014a). More importantly, these strategies may not be practical to implement which could prevent athletes from using $NaHCO_3$. Athletes will not cease training in the days leading to competition for example, meaning that HCO_3^- reserves may be used during these sessions and negate the benefits of chronic and multi-day supplementation. Chronic supplementation also requires higher doses of $NaHCO_3$ (500 $mg \cdot kg^{-1}$ body mass), which results in higher sodium intakes and an excessive number of capsules taken. Ultimately, the efficacy of these protocols to reduce GI side-effects is questionable and may be difficult to implement in practice. In contrast, gastro-resistant $NaHCO_3$ can be acutely ingested and still reduce the prevalence and severity of GI

side-effects (Chapters 4, 5 and 6). Ingesting this supplement 90-120 min before exercise is similar to current recommendations, making it easier for athletes to implement this nutritional strategy.

Gastro-resistant ingestion forms mitigate GI symptoms associated with acute $NaHCO_3$ supplementation. In Chapter 4, the prevalence and severity of GI symptoms was lower when $NaHCO_3$ was administered in delayed-release capsules than as a solution. In Chapter 5, similar reductions were observed with delayed-release $NaHCO_3$ compared to supplementation in gelatine capsules, indicating the gastroprotective effect of minimising bicarbonate release in the stomach. Enteric-coated $NaHCO_3$ were optimal for mitigating GI symptoms post-ingestion and demonstrates *in vivo* that bypassing the stomach is an efficacious model for minimising GI symptoms following $NaHCO_3$ ingestion. Symptoms did not necessarily coincide with peak alkalosis, when exercise would be timed for those adopting an individualised strategy. This finding was later confirmed in Chapter 6, were few participants reported GI symptoms at the onset of exercise. Nonetheless, fewer individuals experienced GI symptoms with enteric-coated $NaHCO_3$, which may therefore benefit those who experience symptoms following supplementation.

Whilst enteric-coated $NaHCO_3$ is already commercially available to athletes, these findings are the first to investigate, and indeed support the use of enteric-coated $NaHCO_3$ to reduce GI side-effects following acute supplementation. The use of enteric-coated $NaHCO_3$ is therefore a valid strategy to attenuate side-effects associated with $NaHCO_3$ supplementation commonly. Athletes should however be made aware that while enteric-coated $NaHCO_3$ can reduce GI side-effects compared with other capsules, this is not consistent between individuals and symptoms may still be experienced at the onset of exercise. Furthermore, given that the type of GI symptoms reported by individuals were not consistent, symptoms may not be

reliable in their presentation. The reliability of enteric-coated $NaHCO_3$ to attenuate GI side-effects therefore warrants further investigation.

7.2.2 Ingestion timing

The experimental chapters of this thesis investigated the time to reach peak blood [HCO_3^-] and pH following the ingestion of 300 mg·kg^{-1} body mass $NaHCO_3$. Figure 7.1 displays the collective findings from these chapters among recreationally trained (n = 26) and trained individuals (n = 11).

Whilst it is typically recommended that $NaHCO_3$ be administered between 60 and 90 min prior to exercise (Renfree, 2007, Price and Simons, 2010, Carr et al. 2011, Siegler et al. 2012), this work demonstrates that 33 out of 37 participants (89%) did not reach peak blood [HCO_3^-] with at least one ingestion form during this window. Furthermore, this thesis presents novel data demonstrating that gastro-resistant $NaHCO_3$ increases the time to induce metabolic alkalosis and reach peak blood [HCO_3^-] and pH. Consequently, in order to maximise blood [HCO_3^-] and pH, existing recommended ingestion timings are not appropriate for gastro-resistant forms of $NaHCO_3$. Instead, delayed-release and enteric-coated $NaHCO_3$ should be consumed earlier than more conventional forms, such as gelatine capsules or solutions. Based on current evidence, athletes supplementing with either delayed-release or enteric-coated $NaHCO_3$ can consume 120 min before exercise to maximise extracellular [HCO_3^-] and pH.

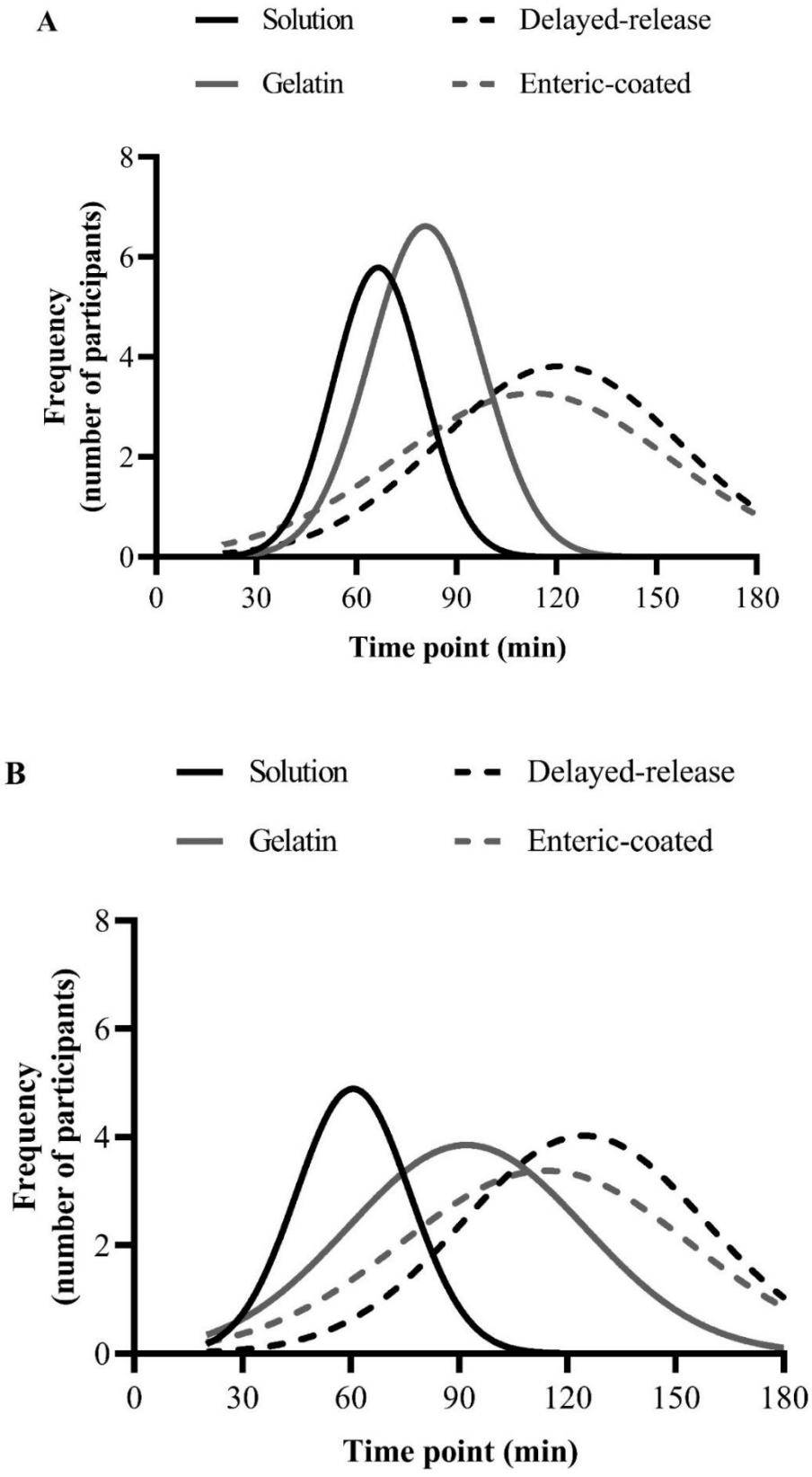

Figure 7.1. Individual time to reach peak blood [HCO_3^-] (A) and pH (B) following 300 mg·kg^{-1} body mass $NaHCO_3$.

Ingestion timings appear to be highly variable between individuals (Chapters 4, 5 and 6). Indeed, the time to reach peak blood [HCO_3^-] and pH varies considerably with gastro-resistant $NaHCO_3$, which is similar to previous work reporting high inter-individual variation with gelatine capsules and solution (Jones et al. 2016, Miller et al. 2016). Furthermore, there appears to be greater variance in the timing of peak blood [HCO_3^-] and pH with gastro-resistant ingestion forms (delayed-release and enteric-coated) compared with either a solution or gelatine capsules (Figure 7.1). While the reasons for these differences are unclear, this may be due to individual differences in gastric emptying or pre-ingestion diet (Davis, Hardy and Fara, 1986) although this warrants further investigation.

7.2.3 Bicarbonate kinetics and other acid–base responses

Bicarbonate kinetics vary considerably between individuals following $NaHCO_3$ supplementation (Jones et al. 2016, Miller et al. 2016). Nevertheless, blood [HCO_3^-] typically increased up to a maximal point in most individuals, followed by a progressive decline due to factors such as reduced absorption and compensatory mechanisms that maintain acid-base balance and homeostasis. Given that gastro-resistant capsules delay the release of $NaHCO_3$, blood [HCO_3^-] is typically elevated for longer compared with gelatine capsules (Chapter 5). This may be beneficial in the context of competition, where it may not be practical time exercise with peak [HCO_3^-]. Furthermore, this may also provide a greater window for $NaHCO_3$ supplementation to enhance performance, although further research is needed.

In Chapters 5 and 6, the increase in blood [HCO_3^-] were blunted with enteric-coated $NaHCO_3$. There are numerous potential reasons for the lower increases observed with enteric-coated compared with gelatine capsules, despite administering the same dose. While the same size and number of capsules were consumed, enteric-coatings delay disintegration of the

capsule, which reduces the time available for absorption (Barbosa, Conway and Merchant, 2017). Another plausible explanation could be due to differences in bicarbonate absorption across the GI tract. Mechanisms responsible for the absorption of bicarbonate vary between the gastric and intestinal compartments, with a shift from passive to active transport (Turnberg et al. 1970). Furthermore, there is some evidence (Turnberg et al. 1970) to suggest that bicarbonate absorption across the intestine is rate limited, meaning that further absorption may not take place irrespective of higher intestinal concentrations of bicarbonate. Although bypassing the stomach may be an efficacious model for minimising GI symptoms following $NaHCO_3$ supplementation, this may not maximise concentrations in the extracellular compartments. Nevertheless, Chapter 6 demonstrates that higher blood [HCO_3^-] do not necessitate greater improvements in high-intensity exercise performance.

7.2.4 Efficacy of enteric-coated sodium bicarbonate to improve exercise performance

Given that enteric-coated $NaHCO_3$ was most effective at attenuating GI symptoms (Chapter 5) the efficacy relating to exercise performance was examined (Chapter 6). Enteric-coated $NaHCO_3$ supplementation was found to improve 4 km TT performance by 2.3 ± 2.6% among trained cyclists. Fewer individuals reported GI symptoms at the onset of exercise with enteric-coated $NaHCO_3$, although the overall severity was similar compared with standard gelatine capsules. Furthermore, enteric-coated $NaHCO_3$ prevented GI symptoms entirely in some individuals, which highlights the potential benefits of this ingestion strategy. Interestingly, increases in blood [HCO_3^-] were lower when $NaHCO_3$ was administered in enteric-coated compared with gelatine capsules, which supports earlier observations from Chapter 5. Despite lowering the bioavailability of circulating [HCO_3^-], enteric-coated $NaHCO_3$ demonstrated similar performance-enhancing effects, with only a slight reduction in the magnitude of effect

compared with gelatine capsules. This finding suggests that higher blood [HCO_3^-] may increase the magnitude of performance-enhancing effects, rather than determine the overall ergogenicity. Enteric-coated $NaHCO_3$ supplementation can therefore be considered a valid strategy to improve high-intensity exercise performance.

7.3 Limitations

A limitation of each study in this thesis is that a sodium-matched placebo was not used as an alternative to cornflour, such as sodium chloride. Given that sodium ingestion can lead to GI disturbances (Gisolfi, 1990), reductions in the prevalence and severity of symptoms may be at least partly attributable to alterations in the release of sodium ions. Nevertheless, the neutralisation of gastric acid is considered the overarching factor contributing to GI symptoms based on previous observations (Gough et al. 2017a, 2018). There are however no currently available ways of ingesting sodium without concomitantly disturbing acid-base balance. Sodium chloride, for example, can decrease blood [HCO_3^-] due to altered shifts in [Cl^-] handling, which may exaggerate the ergogenic effects of $NaHCO_3$ supplementation (Van Montfoort et al. 2004).

A further limitation of each study in this thesis is that the reliability of acid-base responses following $NaHCO_3$ supplementation was not determined. Although blood [HCO_3^-] and pH demonstrate good to excellent test-retest reliability with a solution (Gough et al. 2017a), it is unclear whether acid-base responses are reproducible with capsules. Given that capsules are emptied slowly from the stomach and demonstrate greater variation in gastric emptying (Davis et al. 1986), it is plausible to suggest that the observed acid-base responses and GI symptoms may not be reproducible. Additional research should therefore investigate the test-

retest reliability of acid-base responses and GI symptoms with different $NaHCO_3$ ingestion forms, preferably using a randomised crossover design.

Another potential limitation of the current thesis is that all participants were fasted (~12 h), which may not replicate the digestive state of athletes in practice. Previously, the presence of food in the stomach increased gastric emptying, with even a light breakfast delaying emptying (Davis et al. 1986). While a fasted state controls for the greater variability in gastric emptying after food intake, it is plausible to suggest that acid-base responses and GI symptoms may be affected by digestive state. As such, digestive state should be considered as a potential confounding factor that requires greater control during future studies. Given that gastro-resistant capsules delay the release of $NaHCO_3$, it would be prudent to increase the time (> 3 h) over which acid-base balance and GI symptoms are monitored, particularly in the fed state.

7.4 Future directions

Ingestion form has been shown to have important relevance following $NaHCO_3$ supplementation. Acid-base responses, GI symptoms and exercise performance are all affected by ingestion form, which has often been overlooked in previous studies. This thesis therefore raises several pertinent questions. Firstly, does the ingestion of $NaHCO_3$ as a solution or encased in gelatine capsules alter acid-base responses and exercise performance? If so, previous research that directly compares studies using either solutions or gelatin capsules as equivalent forms of supplementation should be viewed more critically. This could also be added as a factor when performing future meta-analyses to highlight any potential differences between the ingestion forms. Secondly, a study that directly compares the effects of encapsulation alone on acid-base responses, GI symptoms and exercise performance is also warranted. This will help to elucidate the impact that ingesting as a beverage or consuming in capsules has on these determinants of subsequent exercise performance.

The association between enteric-coatings, acid-base responses and GI symptoms following $NaHCO_3$ also poses new questions. Firstly, which enteric formulation optimises the release of $NaHCO_3$ in the small intestine? It appears that different enteric coatings breakdown at different stages across the GI tract, although it is unclear which factor (type of coating, percentage of coating etc.) optimises the uptake of bicarbonate following $NaHCO_3$ supplementation. Secondly, it is unclear whether ingesting $NaHCO_3$ as enteric-coated tablets or capsules would affect the important factors relating to supplementation, such as the timing of ingestion. Simple factors such as the size of the capsule or tablet may influence palatability, acid-base responses and GI symptoms and should not be overlooked. This will be particularly important as more commercially available forms of $NaHCO_3$ are made available to athletes. Finally, whilst GI symptoms may be unpleasant for athletes and may also reduce subsequent exercise performance, no study has investigated the acute and chronic effects of $NaHCO_3$ supplementation on markers of gut damage. This is a pertinent question given that GI symptoms may have additional consequences on gut health and could potentially impact the health of the athlete.

7.5 Conclusions and implications

The outcomes reported in this thesis, as well as the prior literature (Carr, Gore and Dawson, 2011), highlight the high prevalence of GI side-effects with $NaHCO_3$ supplementation. This could indicate why the use of $NaHCO_3$ is low compared with other nutritional ergogenic aids (Knapik et al. 2016) and emphasises the need for alternative ingestion strategies. Whilst existing ingestion strategies, including split- and reduced-dose (e.g. 200 $mg \cdot kg^{-1}$ body mass) protocols can mitigate GI symptoms, the efficacy relating to exercise performance appears to be less robust. Furthermore, there is little consensus as to how these strategies should be implemented in practice and in some cases, they may also be difficult for athletes to implement

(e.g. 4 h ingestion period). Alternatively, bypassing the stomach presents a novel way to attenuate GI symptoms following $NaHCO_3$ ingestion, as has been previously suggested (Farias de Oliveira, Saunders and Artioli, 2018). The current thesis presents the first empirical evidence to suggest that minimising the neutralisation of gastric acid appears to be at least partly achievable using gastro-resistant forms of $NaHCO_3$ (i.e. delayed-release and enteric-coated capsules). Based on the data presented in Chapter 5, enteric-coated $NaHCO_3$ minimises GI side-effects when using an acute loading protocol; a strategy commonly used amongst athletes. While enteric-coated $NaHCO_3$ can reduce bicarbonate bioavailability post-ingestion, this does not necessarily negate the performance-enhancing effects of supplementation. Indeed, the findings of Chapter 6 demonstrate that supplementing with 300 $mg \cdot kg^{-1}$ body mass enteric-coated $NaHCO_3$ can improve performance (~ 2.3%) during a single-bout of high-intensity exercise.

Taken together, the outcomes reported in this thesis demonstrate that enteric-coated $NaHCO_3$ is a valid strategy to mitigate GI symptoms and enhance high-intensity exercise performance. However, given that gastro-resistant $NaHCO_3$ is an emerging ingestion strategy, there is currently less available evidence related to improving exercise performance compared with other ingestion forms. Furthermore, gastro-resistant $NaHCO_3$ is generally more expensive and less commercially available than existing ingestion forms. Based upon these reasons, it is suggested that gastro-resistant $NaHCO_3$ is not a replacement for more conventional ingestion forms (or strategies) but should be viewed as an alternate strategy to reduce GI side-effects among athletes. It is therefore suggested that gastro-resistant $NaHCO_3$ should only be considered among those who report GI side-effects that are not tolerable, despite using existing ingestion forms (i.e. solution and gelatine capsules) and strategies (i.e. split-, multi- and reduced-dose protocols).

Practical recommendations:

- It is recommended that athletes continue to monitor GI responses to $NaHCO_3$ supplementation outside of competition, such as during pre-season or training.
- Athletes supplementing with either solution or gelatine $NaHCO_3$ can consume 60-90 min before exercise to maximise blood [HCO_3^-].
- Athletes supplementing with either delayed-release or enteric-coated $NaHCO_3$ can consume 120 min before exercise to maximise blood [HCO_3^-].
- Delayed-release and enteric-coated $NaHCO_3$ can be used to reduce the risk and severity of GI side-effects following supplementation.
- Enteric-coated $NaHCO_3$ supplementation (300 $mg \cdot kg^{-1}$ body mass) can be used to enhance performance during a single-bout of high-intensity exercise.

References

AHLBORG, B., BERGSTRÖM, J., EKELUND, L.G., GUARNIERI, G., HARRIS, R.C., HULTMAN, E., and NORDESJÖ, L.O., 1972. Muscle metabolism during isometric exercise performed at constant force. *Journal of Applied Physiology*. 33 (2), pp. 224–228.

ALLEN, D.G., LAMB, G.D., and WESTERBLAD, H., 2008. Skeletal muscle fatigue: cellular mechanisms. *Physiological Reviews*. 88 (1), pp. 287–332.

AMANN, M., 2011. Central and peripheral fatigue. *Medicine and Science in Sports and Exercise*. 43 (11), pp. 2039–2045.

AMANN, M., and CALBET, J.A.L., 2008. Convective oxygen transport and fatigue. *Journal of Applied Physiology*. 104 (3), pp. 861–870.

ATHERTON, J.C., 2009. Acid-base balance: maintenance of plasma pH. *Anaesthesia and Intensive Care Medicine*. 10 (11), pp. 557–561.

ATKINSON, G., 2002. Analysis of repeated measurements in physical therapy research: multiple comparisons amongst level means and multi-factorial designs. *Physical Therapy in Sport*. 3, pp. 191–203.

BAKER, S.J., McCORMICK, M.C and ROBERGS, R.A., 2010. Interaction among skeletal muscle metabolic energy systems during intense exercise. *Journal of Nutrition and Metabolism*. 905612, pp. 1–13.

BARBOSA, J.A.C., CONWAY, B.R., and MERCHANT, H.A., 2017. Going Natural: Using polymers from nature for gastro-resistant applications. *British Journal of Pharmacy*. 2 (1), pp. 14–30.

BASSETT, D.R. and HOWLEY, E.T., 2000. Limiting factors for maximum oxygen uptake and determinants of endurance performance. *Medicine and Science in Sports and Exercise*. 31 (1), pp. 70–84.

BELLINGER, P.M., HOWE, S.T., SHING, C.M., and FELL, J.W., 2012. Effect of combined β-alanine and sodium bicarbonate supplementation on cycling performance. *Medicine and Science in Sports and Exercise*. 44 (8), pp. 1545–1551.

BEREND, K., 2013. Acid-base pathophysiology after 130 years: Confusing, irrational and controversial. *Journal of Nephrology*. 26 (2), pp. 254–265.

BILLAT, V.L., SIRVENT, P., PY, G., KORALSZTEIN, J.P., and MERCIER, J., 2003. The concept of maximal lactate steady state: a bridge between biochemistry, physiology and sport science. *Sports Medicine*. 33 (6), pp. 407–426.

BISHOP, D., EDGE, J., DAVIS, C., and GOODMAN, C., 2004. Induced Metabolic Alkalosis Affects Muscle Metabolism and Repeated-Sprint Ability. *Medicine and Science in Sports and Exercise*. 36 (5), pp. 807–813.

BISHOP, D., EDGE, J., THOMAS, C., and MERCIER, J., 2008. Effects of high-intensity training on muscle lactate transporters and postexercise recovery of muscle lactate and hydrogen ions in women. *American Journal of Physiology - Regulatory Integrative and Comparative Physiology*. 295 (6), pp. 1991–1998

BORG, G.A., 1982. Psychophysical bases of perceived exertion. *Medicine and Science in Sports and Exercise*. 14 (5), pp. 377–381.

BREITKREUTZ, J., GAN, T.G., SCHNEIDER, B., and KALISCH, P., 2007. Enteric-coated solid dosage forms containing sodium bicarbonate as a drug substance: an exception from the rule? *Journal of Pharmacy and Pharmacology*. 59 (1), pp. 59–65.

BURKE, L.M. and PYNE, D.B., 2007. Bicarbonate loading to enhance training and competitive performance. *International Journal of Sports Physiology and Performance*. 2 (1), pp. 93–97.

CAIRNS, S.P. and LINDINGER, M.I., 2008. Do multiple ionic interactions contribute to skeletal muscle fatigue? *Journal of Physiology*. 586 (17), pp. 4039–4054.

CALLAHAN, M.J., PARR, E.B., HAWLEY, J.A., and BURKE, L.M., 2017. Single and combined effects of beetroot crystals and sodium bicarbonate on 4-km cycling time trial performance. *International Journal of Sport Nutrition and Exercise Metabolism*. 27 (3), pp. 271–278.

CAMERON, S.L., MCLAY-COOKE, R.T., BROWN, R.C., GRAY, A.R., and FAIRBAIRN, K.A., 2010. Increased blood pH but not performance with sodium bicarbonate supplementation in elite rugby union players. *International Journal of Sport Nutrition*. 20 (4), pp. 307–321.

CARR, A.J., GORE, C.J., and DAWSON, B., 2011. Induced alkalosis and caffeine supplementation: Effects on 2,000-m rowing performance. *International Journal of Sport Nutrition and Exercise Metabolism*. 21 (5), pp. 357–364.

CARR, A.J., HOPKINS, W.G., and GORE, C.J., 2011. Effects of acute alkalosis and acidosis on performance: A meta-analysis. *Sports Medicine*. 41 (10), pp. 801–814.

CARR, A.J., SLATER, G.J., GORE, C.J., DAWSON, B., and BURKE, L.M., 2011. Effect of sodium bicarbonate on [HCO3-], pH, and gastrointestinal symptoms. *International Journal of Sport Nutrition and Exercise Metabolism*. 21 (3), pp. 189–194.

CARTER, J. and JEUKENDRUP, A.E., 2002. Validity and reliability of three commercially available breath-by-breath respiratory systems. *European Journal of Applied Physiology*. 86 (5), pp. 435–441.

CHRISTENSEN, P.M., SHIRAI, Y., RITZ, C., and NORDSBORG, N.B., 2017. Caffeine and bicarbonate for speed. A meta-analysis of legal supplements potential for improving intense endurance exercise performance. *Frontiers in Physiology*. 8 (5), pp. 1–16.

COHEN, J., 1998. *Statistical Power Analysis for the Behavioral Sciences*. 2nd ed. Hillsdale (NJ): Lawrence Erlbaum Associates pp. 567.

CORREIA-OLIVEIRA, C.R., LOPES-SILVA, J.P., BERTUZZI, R., MCCONELL, G.K., BISHOP, D.J., LIMA-SILVA, A.E., and KISS, M.A.P.D.M., 2017. Acidosis, but Not

Alkalosis, Affects Anaerobic Metabolism and Performance in a 4-km Time Trial. *Medicine and Science in Sports and Exercise*. 49 (9), pp. 1899–1910.

COSTILL, D.L., BARNETT, A., SHARP, R., FINK, W.J., and KATZ, A., 1983. Leg muscle pH following sprint running. *Medicine and Science in Sports and Exercise*. 15 (4), pp. 325–329.

COSTILL, D.L., DANIELS, J., EVANS, W., FINK, W., KRAHENBUHL, G., and SALTIN, B., 1976. Skeletal muscle enzymes and fiber composition in male and female track athletes. *Journal of Applied Physiology*. 40 (2), pp. 149–154.

COSTILL, D.L., VERSTAPPEN, F., KUIPERS, H., JANSSEN, E., and FINK, W., 1984. Acid-base balance during repeated bouts of exercise: influence of HCO3. *International Journal of Sports Medicine*. 5 (5), pp. 228–231.

DAVIS, S.S., HARDY, J.G., and FARA, J.W., 1986. Transit of pharmaceutical dosage forms through the small intestine. *Gut*. 27 (8), pp. 886–892.

DE ARAUJO DIAS, G.F., EIRA SILVA, V. Da, PAINELLI, V.D.S., SALE, C., ARTIOLI, G.G., GUALANO, B., and SAUNDERS, B., 2015. (In)consistencies in responses to sodium bicarbonate supplementation: A randomised, repeated measures, counterbalanced and double-blind study. *PLoS ONE*. 10 (11), pp. 1–13.

DEB, S.K., GOUGH, L.A., SPARKS, S.A., and MCNAUGHTON, L.R., 2018. Sodium bicarbonate supplementation improves severe-intensity intermittent exercise under moderate acute hypoxic conditions. *European Journal of Applied Physiology*. 118 (3), pp. 607–615.

DENNIG, H., TALBOTT, J.H., EDWARDS, H.T., and DILL, D.B., 1931. Effect of Acidosis and Alkalosis Upon Capacity for Work. *The Journal of Clinical investigation*. 9 (4), pp. 601–13.

DEPAUW, K., ROELANDS, B., GEUS, B. DE, and MEEUSEN, R., 2013. Guidelines to classify subject groups in sport- science research. *International Journal of Sports Physiology and Performance*. 8 (2), pp. 111–122.

DILL, D.B., EDWARDS, H.T., and TALBOT, J.H., 1932. Alkalosis and the capacity for work. *Journal of Biology and Chemistry*. 91 (1), pp. 58–59

DONALDSON, S.K., HERMANSEN, L., and BOLLES, L., 1978. Differential, direct effects of H+ on Ca2+ activated force of skinned fibers from the soleus, cardiac and adductor magnus muscles of rabbits. *Pflügers Archiv*. 376 (1), pp. 55–65.

DRILLER, M.W., GREGORY, J.R., WILLIAMS, A.D., and FELL, J.W., 2012. The effects of serial and acute nahco3 loading in well-trained cyclists. *Journal of Strength and Conditioning Research*. 26 (10), pp. 2791–2797.

ENOKA, R.M., and STUART, D.G., 1992. Neurobiology of muscle fatigue. *Journal of Applied Physiology*. 72 (5), pp. 1631–1648.

FABIATO, A., and FABIATO, F., 1978. Effects of pH on the myofilaments and the sarcoplasmic reticulum of skinned cells from cardiace and skeletal muscles. *The Journal of Physiology*. 276 (1), pp. 233–255.

FARIAS DE OLIVEIRA, L., SAUNDERS, B., and ARTIOLI, G., 2018. Is By-passing the Stomach a Means to Optimise Sodium Bicarbonate Supplementation? A Case-study With a Post-Bariatric Surgery Individual. *International Journal of Sport Nutrition and Exercise Metabolism*. 28 (6), pp. 1–44.

FAUL, F., ERDFELDER, E., LANG, A.., and BUCHNER, A., 2007. G*Power 3: A flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behaviour Research Methods*. 39 (2), pp. 175–191.

FITTS, R.H., 1994. Cellular mechanisms of muscle fatigue. *Physiological Reviews*. 74 (1), pp. 49–94.

FITTS, R.H., 2016. The role of acidosis in fatigue: Pro perspective. *Medicine and Science in Sports and Exercise*. 48 (11), pp. 2335–2338.

GANDEVIA, S.C., 2001. Spinal and Supraspinal Factors in Human Muscle Fatigue. *Physiological Reviews*. 81 (4), pp. 1725–1789.

GISOLFI, C.., 1990. Human intestinal water absorption: direct vs. indirect measurements. *American Journal of Physiology - Cell Physiology*. 258, pp. 216–222.

DI GIULIO, C.D., DANIELE, F., and TIPTON, C.M., 2006. Angelo Mosso and muscular fatigue: 116 years after the first congress of physiologists: IUPS commemoration. *AJP: Advances in Physiology Education*. 30 (2), pp. 51–57.

GOLDFINCH, J., MC NAUGHTON, L., and DAVIES, P., 1988. Induced metabolic alkalosis and its effects on 400-m racing time. *European Journal of Applied Physiology and Occupational Physiology*. 57 (1), pp. 45–48.

GOUGH, L.A., DEB, S.K., SPARKS, A., and MCNAUGHTON, L.R., 2017a. The Reproducibility of 4-km Time Trial (TT) Performance Following Individualised Sodium Bicarbonate Supplementation: a Randomised Controlled Trial in Trained Cyclists. *Sports Medicine - Open.* 3 (1), pp. 1–10

GOUGH, L.A., DEB, S.K., SPARKS, A.S., and MCNAUGHTON, L.R., 2017b. The Reproducibility of Blood Acid Base Responses in Male Collegiate Athletes Following Individualised Doses of Sodium Bicarbonate: A Randomised Controlled Crossover Study. *Sports Medicine*. 47 (10), pp. 2117–2127.

GOUGH, L.A., DEB, S.K., SPARKS, S.A., and MCNAUGHTON, L.R., 2018. Sodium bicarbonate improves 4 km time trial cycling performance when individualised to time to peak blood bicarbonate in trained male cyclists. *Journal of Sports Sciences*. 36 (15), pp. 1705–1712.

GRAFEN, A. and HAILS, R., 2002. Modern statistics for the life sciences. Oxford, UK: Oxford University Press, pp 368.

GUYTON, A.C., 1976. Textbook of medical physiology. Philadelphia: Saunders.

HEIBEL, A.B., PERIM, P.H.L., OLIVEIRA, L.F., MCNAUGHTON, L.R., and SAUNDERS, B., 2018. Time to Optimize Supplementation: Modifying Factors Influencing the Individual Responses to Extracellular Buffering Agents. *Frontiers in Nutrition*. 5 (5), pp. 1–12.

HERMANSEN, L. and OSNES, J.B., 1972. Blood and muscle pH after maximal exercise in man. *Journal of Applied Physiology*. 32 (3), pp. 304–308.

HIGGINS, M.F., JAMES, R.S., and PRICE, M.J., 2013. The effects of sodium bicarbonate (NaHCO3) ingestion on high intensity cycling capacity. *Journal of Sports Sciences*. 31 (9), pp. 972–981.

HILL, A. V, LONG, C.N.H., and LUPTON, H., 1924a. Muscular Exercise, Lactic Acid, and the Supply and Utilisation of Oxygen: Parts I-III. *Proceedings of the Royal Society of London B Biological Sciences*. 96 (679), pp. 438–475.

HILL, A. V, LONG, C.N.H., and LUPTON, H., 1924b. Muscular Exercise, Lactic Acid, and the Supply and Utilisation of Oxygen: Parts IV-VI. *Proceedings of the Royal Society of London B Biological Sciences*. 97, pp. 84–138.

HILL, A. V, LONG, C.N.H., and LUPTON, H., 1924c. Muscular Exercise, Lactic Acid, and the Supply and Utilisation of Oxygen: Parts VII-VIII. *Proceedings of the Royal Society of London B Biological Sciences*. 97, pp. 155–176.

HILL, A. V and LUPTON, H., 1923. Muscular exercise, lactic acid, and the supply and utilisation of oxygen. *Quarterly Journal of Medicine*. 16, pp. 135–171.

HILTON, N.P., LEACH, N.K., HILTON, M.M., SPARKS, A., and MCNAUGHTON, L.R., 2019. Enteric-Coated Sodium Bicarbonate Attenuates Gastrointestinal Side-Effects. *International Journal of Sport Nutrition and Exercise Metabolism*. 21, pp. 62–68.

HILTON, N.P., LEACH, N.K., SPARKS, S.A., GOUGH, L.A., CRAIG, M.M., DEB, S.K., and MCNAUGHTON, L.R., 2019. A Novel Ingestion Strategy for Sodium Bicarbonate

Supplementation in a Delayed-Release Form: a Randomised Crossover Study in Trained Males. *Sports Medicine - Open*. 5 (1), pp. 1–10.

HOLLIDGE-HORVAT, M.G., PAROLIN, M.L., WONG, D., JONES, N.L., and HEIGENHAUSER, G.J.F., 2000. Effect of induced metabolic alkalosis on human skeletal muscle metabolism during exercise. *American Journal of Physiology - Endocrinology and Metabolism*. 278 (2 41-2), pp. 316–329.

JONES, N.L., SUTTON, J.R., TAYLOR, R., and TOEWS, C.J., 1977. Effect of pH on cardiorespiratory and metabolic responses to exercise. *Journal of Applied Physiology*. 43 (6), pp. 959–964.

JONES, R.L., STELLINGWERFF, T., ARTIOLI, G.G., SAUNDERS, B., COOPER, S., and SALE, C., 2016. Dose-response of sodium bicarbonate ingestion highlights individuality in time course of blood analyte responses. *International Journal of Sport Nutrition and Exercise Metabolism*. 26 (5), pp. 445–453.

JOYNER, M.J. and COYLE, E.F., 2008. Endurance exercise performance: The physiology of champions. *Journal of Physiology*. 586 (1), pp. 35–44.

KAHLE, L.E., KELLY, P.V, ELIOT, K.A., and WEISS, E.P., 2013. Acute sodium bicarbonate loading has negligible effects on resting and exercise blood pressure but causes gastrointestinal distress. *Nutrition Research*. 33 (6), pp. 479–486.

KENT-BRAUN, J.A., FITTS, R.H., and CHRISTIE, A., 2012. Skeletal muscle fatigue. *Journal of Comparative Physiology*. 97, pp. 997–1044.

KINNEAR, P. and GRAY, L., 1995. SPSS for Windows made simple.

KNAPIK, J.J., STEELMAN, R.A., HOEDEBECKE, S.S., AUSTIN, K.G., FARINA, E.K., and LIEBERMAN, H.R., 2016. Prevalence of Dietary Supplement Use by Athletes: Systematic Review and Meta-Analysis. *Sports Medicine*. 46 (1), pp. 103–123.

KNUTH, S.T., DAVE, H., PETERS, J.R., and FITTS, R.H., 2006. Low cell pH depresses peak power in rat skeletal muscle fibres at both 30°C and 15°C: Implications for muscle fatigue. *Journal of Physiology*. 575 (3), pp. 887–899.

KOWALCHUK, J.M., HEIGENHAUSER, J.G., and JONES, N.L., 1984. Effect of pH on metabolic and cardiorespiratory responses during progressive exercise. *Journal of Applied Physiology: Respiratory, Environmental and Exercise Physiology*. 57 (5), p. 1558–1563.

LAKENS, D., 2013. Calculating and reporting effect sizes to facilitate cumulative science: a practical primer for t-tests and ANOVAs. *Frontiers in Psychology*. 4, pp. 1–12.

LANCHA JUNIOR, A.H., DE SALLES PAINELLI, V., SAUNDERS, B., and ARTIOLI, G.G., 2015. Nutritional Strategies to Modulate Intracellular and Extracellular Buffering Capacity During High-Intensity Exercise. *Sports Medicine*. 45 (1), pp. 71–81.

LAURSEN, P.B., and JENKINS, D.G., 2002. The scientific basis for high-intensity interval training: optimising training programmes and maximising performance in highly trained endurance athletes. *Sports Medicine*. 32 (1), pp. 53–73.

MACLAREN, D.P.M., GIBSON, H., PARRY-BILLINGS, M., and EDWARDS, R.H.T., 1989. A review of metabolic and physiological factors in fatigue. *Exercise and Sports Science Reviews.* 17, pp. 29–66.

MACLAREN, D.P.M., and MORTON, J., 2012. Biochemistry for sport and exercise metabolism. Sussex, UK: John Wiley and Sons.

MAINWOOD, G.W. and CECHETTO, D., 1980. The effect of bicarbonate concentration on fatigue and recovery in isolated rat diaphragm muscle. *Canadian Journal of Physiology and Pharmacology*. 58 (6), pp. 624–632.

MAINWOOD, G.W. and WORSLEY-BROWN, P., 1975. Effects of extraceullular pH and buffer concentration on the efflux of lactate from frog sartorius muscle. *Journal of Physiology*. 250, pp. 1–22.

MARCORA, S.M. and STAIANO, W., 2010. The limit to exercise tolerance in humans: Mind over muscle? *European Journal of Applied Physiology*. 109 (4), pp. 763–770.

MARZORATI, M., POSSEMIERS, S., VERHELST, A., CADÉ, D., MADIT, N., and VAN DE WIELE, T., 2015. A novel hypromellose capsule, with acid resistance properties, permits the targeted delivery of acid-sensitive products to the intestine. *LWT - Food Science and Technology*. 60 (1), pp. 544–551.

MATSON, L.G. and TRAN, Z.V., 1993. Effects of Sodium Bicarbonate Ingestion on Anaerobic Performance: A Meta-Analytic Review. *International Journal of Sport Nutrition*. 3 (1), pp. 2–28.

MCCARTNEY, N., HEIGENHAUSER, G.J., and JONES, N.L., 1983. Effects of pH on maximal power output and fatigue during short-term dynamic exercise. *Journal of Applied Physiology: Respiratory, Environmental and Exercise Physiology*. 55, pp. 225–259.

MCNAUGHTON, L.R., 1992. Bicarbonate ingestion: Effects of dosage on 60 s cycle ergometry. *Journal of Sports Sciences*. 10 (5), pp. 415–423.

MCNAUGHTON, L.R., GOUGH, L., DEB, S., BENTLEY, D., and SPARKS, S.A., 2016. Recent Developments in the Use of Sodium Bicarbonate as an Ergogenic Aid. *Current Sports Medicine Reports*. 15 (4), pp. 233–244.

MCNAUGHTON, L.R., SIEGLER, J., and MIDGLEY, A., 2008. Ergogenic Effects of Sodium Bicarbonate. *Current Sports Medicine Reports*. 7 (4), pp. 230–236.

MCNAUGHTON, L.R. and THOMPSON, D., 2001. Acute versus chronic sodium bicarbonate ingestion and anaerobic work and power output. *The Journal of Sports Medicine and Physical Fitness*. 41 (4), pp. 456–462.

MESSONNIER, L., KRISTENSEN, M., JUEL, C., and DENIS, C., 2007. Importance of pH regulation and lactate/H+ transport capacity for work production during supramaximal exercise in humans. *Journal of Applied Physiology*. 102, pp. 1936–1944.

MICKLEWRIGHT, D., ST CLAIR GIBSON, A., GLADWELL, V., and AL SALMAN, A., 2017. Development and Validity of the Rating-of-Fatigue Scale. *Sports Medicine*. 47 (11), pp. 2375–2393.

MIDGLEY, A.W., MCNAUGHTON, L.R., POLMAN, R., and MARCHANT, D., 2007. Criteria for determination of maximal oxygen uptake: A brief critique and recommendations for future research. *Sports Medicine*. 37 (12), pp. 1019–1028.

MILLER, P., ROBINSON, A.L., SPARKS, S.A., BRIDGE, C.A., BENTLEY, D.J., and MCNAUGHTON, L.R., 2016. The Effects of Novel Ingestion of Sodium Bicarbonate on Repeated Sprint Ability. *Journal of Strength and Conditioning Research*. 30 (2), pp. 561–568.

MITCHELL, R.A., LOESCHCKE, H.H., MASSION, W.H., and SEVERINGHAUS, J.W., 1963. Respiratory responses mediated through superficial chemosensitive areas on the medulla. *Journal of Applied Physiology*. 18 (3), pp. 523–533.

MAUGHAN, R.J., BURKE, L.M., DVORAK, J., ET AL., 2018. IOC consensus statement: dietary supplements and the high-performance athlete. *British Journal of Sports Medicine*. 52 (7), pp. 439–455.

VAN MONTFOORT, M.C.E., VAN DIEREN, L., HOPKINS, W.G., and SHEARMAN, J.P., 2004. Effects of ingestion of bicarbonate, citrate lactate, and chloride on sprint running. *Medicine and Science in Sports and Exercise*. 36 (7), pp. 1239–1243.

MOSSO, A., 1915. Fatigue. 3rd ed. London: Allen and Unwin.

MUELLER, S.M., GEHRIG, S.M., FRESE, S., WAGNER, C.A., BOUTELLIER, U., and TOIGO, M., 2013. Multiday acute sodium bicarbonate intake improves endurance capacity and reduces acidosis in men. *Journal of the International Society of Sports Nutrition*. 10 (1), pp. 1–9.

PEART, D.J., MCNAUGHTON, L.R., MIDGLEY, A.W., TAYLOR, L., TOWLSON, C., MADDEN, L.A., and VINCE, R. V, 2011. Pre-exercise alkalosis attenuates the heat shock

protein 72 response to a single-bout of anaerobic exercise. *Journal of Science and Medicine in Sport*. 14 (5), pp. 435–440.

POUPIN, N., CALVEZ, J., LASSALE, C., CHESNEAU, C., and TOMÉ, D., 2012. Impact of the diet on net endogenous acid production and acid-base balance. *Clinical Nutrition*. 31 (3), pp. 313–321.

PRICE, M.J. and SIMONS, C., 2010. The effect of sodium bicarbonate ingestion on high-intensity intermittent running and subsequent performance. *Journal of Strength and Conditioning Research*. 24 (7), pp. 1834–1842.

PYNE, D.B., BOSTON, T., MARTIN, D.T., and LOGAN, A., 2000. Evaluation of the Lactate Pro blood lactate analyser. *European Journal of Applied Physiology*. 82 (1–2), pp. 112–116.

RAYMER, G.H., 2004. Metabolic effects of induced alkalosis during progressive forearm exercise to fatigue. *Journal of Applied Physiology*. 96 (6), pp. 2050–2056.

REILLY, T., 1990. Human circadian rhythms and exercise. *Critical Reviews in Biomedical Engineering*. 18 (3), pp. 165–180.

RENFREE, A., 2007. The time course for changes in plasma [h+] after sodium bicarbonate ingestion. *International Journal of Sports Physiology and Performance*. 2 (3), pp. 323–326.

ROTH, D.A., 1991. The sarcolemmal lactate transporter: transmembrane determinants of lactate flux. *Medicine and Science in Sports and Exercise*. 23 (8), pp. 925–934.

RUBANA, I.E. and AULIK, I. V, 1989. Buffer function of hemoglobin. *Sports Medicine, Training and Rehabilitation*. 1 (2), pp. 125–126.

SAHLIN, K., 1992. Metabolic factors in fatigue. *Sports Medicine*. 13 (2), pp. 99–107.

SAHLIN, K., HARRIS, R.C., and HULTMAN, E., 1975. Creatine kinase equilibrium and lactate content compared with muscle pH in tissue samples obtained after isometric exercise. *Biochemical Journal*. 152 (2), pp. 173–180.

SALE, C., SAUNDERS, B., HUDSON, S., WISE, J.A., HARRIS, R.C., and SUNDERLAND, C.D., 2011. Effect of β-alanine plus sodium bicarbonate on high-intensity cycling capacity. *Medicine and Science in Sports and Exercise*. 43 (10), pp. 1972–1978.

SAUNDERS, B., SALE, C., HARRIS, R.C., and SUNDERLAND, C., 2014a. Sodium bicarbonate and high-intensity-cycling capacity: Variability in responses. *International Journal of Sports Physiology and Performance*. 9 (4), pp. 627–632.

SAUNDERS, B., SALE, C., HARRIS, R.C., and SUNDERLAND, C., 2014b. Effect of sodium bicarbonate and beta-alanine on repeated sprints during intermittent exercise performed in hypoxia. *International Journal of Sport Nutrition and Exercise Metabolism*. 24 (2), pp. 196–205.

SCHILLINGS, M.L., HOEFSLOOT, W., STEGEMAN, D.F., ZWARTS, M.J., 2003. Relative contributions of central and peripheral factors to fatigue during a maximal sustained effort. *European Journal of Applied Physiology*. 90 (5-6), pp. 562–568

SIEGLER, J.C. and MARSHALL, P., 2015. The effect of metabolic alkalosis on central and peripheral mechanisms associated with exercise-induced muscle fatigue in humans. *Experimental Physiology*. 100 (5), pp. 519–530.

SIEGLER, J.C., MARSHALL, P.W.M., BRAY, J., and TOWLSON, C., 2012. Sodium bicarbonate supplementation and ingestion timing: Does it matter? *Journal of Strength and Conditioning Research*. 26 (7), pp. 1953–1958.

SIEGLER, J.C., MIDGLEY, A.W., POLMAN, R.C.J., and LEVER, R., 2010. Effects of various sodium bicarbonate loading protocols on the time-dependent extracellular buffering profile. *Journal of Strength and Conditioning Research*. 24 (9), pp. 2551–2557.

SMITH, J.L., MARTIN, P.G., GANDEVIA, S.C., and TAYLOR, J.L., 2007. Sustained contraction at very low forces produces prominent suprasinal fatigue in human elbow flexor muscles. *Journal of Applied Physiology*. 103 (2), pp. 560–568.

SOGAARD, K., GANDEVIA, S.C., TODD, G., PETERSEN, N.T., TAYLOR, J.L., 2006. The effects of sustained low-intensity contractions on suprapinal fatigue in human elbow flexor muscles. *Journal of Physiology*. 573 (2), pp. 511–523.

SPRIET, L.L., MATSOS, C.G., and PETERS, S.J., 1985. Effects of acidosis on rat muscle metabolism and performance during heavy exercise. *American Journal of Physiology - Cell Physiology*. 17 (2), pp. 833–839.

ST CLAIR GIBSON, A. and NOAKES, T.D., 2004. Evidence for complex system integration and dynamic neural regulation of skeletal muscle recruitment during exercise in humans. *British Journal of Sports Medicine*. 38 (6), pp. 797–806.

STADLBAUER, V., WALLNER, S., STOJAKOVIC, T., and SMOLLE, K.H., 2011. Comparison of 3 different multianalyte point-of-care devices during clinical routine on a medical intensive care unit. *Journal of Critical Care*. 26 (4), pp. 433.

SUTTON, J.R., JONES, N.L., and TOEWS, C.J., 1981. Effect of pH on Muscle Glycolysis during Exercise. *Clinical Science*. 61 (3), pp. 331–338.

THOMAS, K., GOODALL, S., STONE, M., HOWATSON, G., GIBSON, A.S.C., and ANSLEY, L., 2014. Central and peripheral fatigue in male cyclists after 4-, 20-, and 40-km time trials. *Medicine and Science in Sports and Exercise*. 47 (3), pp. 537–546.

THOMPSON, B., 2007. Effect sizes, confidence intervals, and confidence intervals for effect sizes. *Psychology in the Schools*. 44, pp. 423–432.

TURNBERG, L.A., FORDTRAN, J.S., CARTER, N.W., and RECTOR, F.C., 1970. Mechanism of bicarbonate absorption and its relationship to sodium transport in the human jejunum. *The Journal of clinical investigation*. 49 (3), pp. 548–556.

WESTERBLAD, H., 2016. Acidosis is not a significant cause of skeletal muscle fatigue. *Medicine and Science in Sports and Exercise*. 48 (11), pp. 2339–2342.

WESTERTERP-PLANTENGA, M., DIEPVENS, K., JOOSEN, A.M.C.P., BÉRUBÉ-PARENT, S., and TREMBLAY, A., 2006. Metabolic effects of spices, teas, and caffeine. *Physiology and Behaviour*. 89 (1), pp. 85–91.

WESTSTRATE, J.A., WUNNINK, I., DEURENBERG, P., and HAUTVAST, J.G.A.J., 1990. Alcohol and its acute effects on resting metabolic rate and diet-induced thermogenesis. *British Journal of Nutrition*. 64 (2), pp. 413–425.

WILKES, D., GLEDHILL, N., and SMYTH, R., 1983. Effect of acute induced metabolic alkalosis on 800-m racing time. *Medicine and Science in Sports and Exercise*. 15 (4), pp. 277–280.